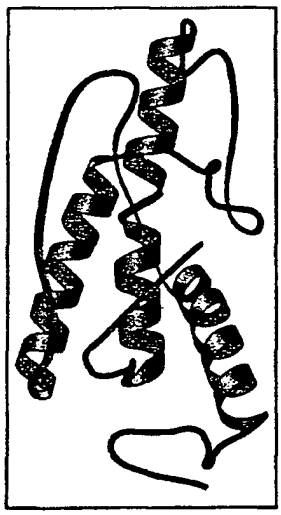
DNA REPAIR AND MUTAGENESIS

ERROL C. FRIEDBERG
Department of Pathology, The University of Texas Southwestern Medical Center, Dallas, Texas

GRAHAM C. WALKER
Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts

WOLFRAM SIEDE
Department of Pathology, The University of Texas Southwestern Medical Center, Dallas, Texas



ASM Press
Washington, D.C.

Library of Congress Cataloging-in-Publication Data

Friedberg, Errol C.
DNA repair and mutagenesis / Errol C. Friedberg, Graham C. Walker, Wolfram Siede.
p. cm.
Includes bibliographical references and index.
ISBN 1-55581-088-8
1. DNA repair. 2. Mutagenesis. I. Walker, Graham C. II. Siede, Wolfram. III. Title.
[DNLM: 1. DNA Damage. 2. DNA Repair. 3. Mutagenesis. QH 465 F899d 1955]
QH467.F753 1995
574.87'328—dc20
DNLM/DLC
for Library of Congress 94-43983
CIP

10 9 8 7 6 5 4 3 2 1

Cover illustration: Proposed structure of the phage T4 PD-DNA glycosylase (endonuclease V or DenV protein). (*See Figure 4–24 on page 167.*)

5

Nucleotide Excision Repair in Prokaryotes

UvrABC Damage-Specific Endonuclease of *E. coli* 192
Postincisional Events—Excision of Damaged Nucleotides, Repair Synthesis, and DNA Ligation 210
Nucleotide Excision Repair of Cross-Links in DNA—A Special Situation 214
Coupling of Transcription and Nucleotide Excision Repair in *E. coli* 216
Miscellaneous Functions Possibly Associated with Nucleotide Excision Repair 218
Detection and Measurement of Nucleotide Excision Repair 219
Nucleotide Excision Repair in Other Prokaryotes 223
Summary and Conclusions 225

Years before the discovery of DNA glycosylases, apurinic/apyrimidinic (AP) endonucleases, and the general phenomenon of base excision repair, the most extensively studied model lesion for excision repair was the pyrimidine dimer, a major photoproduct produced in DNA by UV radiation at ~254 nm (see chapter 1). This form of DNA damage is very convenient to study, since it is easy to generate in the laboratory (requiring only a germicidal lamp), it is chemically stable in DNA, and reliable and sensitive methods exist for its detection and quantitation (48, 49). Additionally, it is a biologically relevant form of DNA damage because the same type of base damage is produced in vivo when cells are exposed to sunlight (see chapter 1).

It has long been known that strains of *Escherichia coli* exposed to UV radiation undergo recovery processes which involve the repair of DNA containing pyrimidine dimers (47, 69, 95). Such recovery can be demonstrated as an increased survival of the bacteria themselves or as their ability to promote the survival of UV-irradiated bacteriophages for which the bacteria serve as hosts. The latter phenomenon is called *host cell reactivation* and is a sensitive indicator of DNA repair, both in prokaryotes and in mammalian cells infected with viruses (47, 69, 95). More recently it has become appreciated that the repair of a second major photoproduct in DNA [the so-called (6-4) lesion (see chapter 1)] is also important for biological recovery from UV radiation.

Following the discovery of enzymatic photoreactivation (see chapter 3), DNA repair modes associated with recovery from the effects of UV radiation were distinguished as *light repair* (to indicate the dependence of photoreactivation on visible light) and *dark repair* (to indicate DNA repair processes which are independent of light). During the early 1960s a series of experiments reported by Setlow and Carrier (176) and by Boyce and Howard-Flanders (17) led to important insights into the nature of dark repair of UV radiation-induced damage. Following the irradiation of UV-resistant (wild-type) and UV-sensitive (mutant) *E. coli* strains,

it was noted that in the wild-type strains pyrimidine dimers were lost from the high-molecular-weight (acid-insoluble) fraction of DNA and appeared in the low-molecular-weight (acid-soluble) phase during post-UV incubation. The kinetics of this process correlated well with the resumption of DNA synthesis (178) and led to the suggestion that the loss of dimers from DNA (i.e., their *excision*) was directly related to the recovery of replicative DNA synthesis and hence to survival. This hypothesis was supported by the observation that in mutant UV radiation-sensitive and host-cell reactivation-defective (*hcr*) strains, thymine-containing dimers were not lost from high molecular weight DNA during post-UV incubation in the absence of photoreactivating light.

These seminal observations, together with the demonstration by Pettijohn and Hanawalt (136, 137) of repair synthesis of DNA in cells exposed to UV radiation, defined the process that is called *nucleotide excision repair*, a process by which damaged bases such as pyrimidine dimers and (6-4) photoproducts are enzymatically excised from DNA as intact nucleotides (actually as part of *oligonucleotide fragments*) rather than as free bases. Like base excision repair, nucleotide excision repair is a multistep process which eventually leads to the formation of a gap in the DNA duplex. This gap is filled by repair synthesis and covalently sealed by DNA ligase, as discussed in chapter 4. However, nucleotide excision repair is a biochemically more complex process than base excision repair and involves many more gene products, particularly in eukaryotic cells. This chapter is devoted to a consideration of nucleotide excision repair in prokaryotes, in which *E. coli* will once again constitute the primary paradigm. The next three chapters are devoted to a consideration of nucleotide excision repair in lower eukaryotes and in mammalian cells.

UvrABC Damage-Specific Endonuclease of *E. coli*

Early genetic analyses identified three loci, designated *uvrA*$^+$, *uvrB*$^+$ and *uvrC*$^+$, that are required for the excision of pyrimidine dimers in *E. coli* (73, 74, 105, 209). Subsequently, two other genes, *uvrD*$^+$ and *polA*$^+$, were implicated in this process as well (152, 154, 210). We have already encountered the *polA*$^+$ gene, which encodes DNA polymerase I (PolI), in the context of the discussion of repair synthesis during base excision repair (see chapter 4). The use of techniques (some of which are discussed below) that reveal the presence of single-strand breaks (nicks) in DNA established that the *uvrA*$^+$, *uvrB*$^+$, and *uvrC*$^+$ genes are specifically required for *endonucleolytic incision of DNA* containing pyrimidine dimers (145, 182). These observations reinforced the notion that the dark repair of pyrimidine dimers does indeed occur by an excision-resynthesis mode and that this must somehow involve breakage of the polynucleotide chain specifically at or near sites of pyrimidine dimers; i.e., the DNA must somehow be subject to *damage-specific incision*.

One mechanism for the specific incision of DNA containing pyrimidine dimers that was discussed in chapter 4 is by the sequential action of a pyrimidine dimer-DNA glycosylase (PD-DNA glycosylase) and an AP endonuclease. However, DNA glycosylase/AP endonuclease-mediated incision of DNA containing pyrimidine dimers is a highly specialized form of base excision repair which is confined to *Micrococcus luteus* and to *E. coli* infected with bacteriophage T4 and possibly to the yeast *Saccharomyces cerevisiae*. The stringent substrate specificity of the T4 PD-DNA glycosylase is consistent with the observation that phage T4 mutants which are defective in the *denV*$^+$ gene (which encodes this DNA glycosylase) are not abnormally sensitive to any other type of base damage. On the other hand, it is well established that *E. coli* mutants defective in the *uvrA*$^+$, *uvrB*$^+$, or *uvrC*$^+$ gene(s) are sensitive not only to UV radiation but also to a diverse array of chemical agents, including mitomycin, nitrogen mustard, photoactivated psoralen, and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (73, 74, 105, 115), to name just a few (Fig. 5–1).

Hence, a reasonable answer to the question why *E. coli* and many other organisms do not possess a PD-DNA glycosylase to deal with the potentially lethal

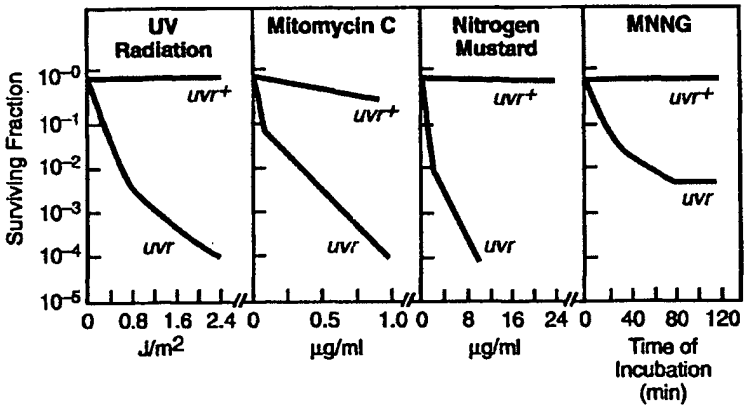


Figure 5–1 Nucleotide excision repair-defective (*uvr*) strains of *E. coli* are abnormally sensitive to killing by a wide variety of DNA-damaging agents, including UV radiation, mitomycin, nitrogen mustard, and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). (*Adapted from Friedberg [45a] with permission.*)

and mutagenic effects of pyrimidine dimers is that the presence of many other types of base damage that challenged the survival of cells during biological evolution provided the selection for a more-general excision repair mode which could serve to identify these multiple types of damage and effect their removal from DNA. As discussed here and in subsequent chapters, we still do not understand precisely how the nucleotide excision repair machinery is able to identify diverse types of base damage which apparently have few chemical and even structural features in common. Nor is it fully understood how this machinery is able to specifically recognize such damage in the general background of other perturbations in DNA structure which naturally arise from its dynamic state and its participation as a substrate in other aspects of DNA metabolism.

Although biological studies on nucleotide excision repair in *E. coli* were initiated over 30 years ago, it is only in the last decade that major insights have been obtained concerning the detailed biochemistry of this process. Several factors impeded progress in this area of biochemistry. For one thing, all cells, including *E. coli*, contain multiple nuclease activities which rapidly degrade DNA in cell extracts. The preparation of cell extracts by Seeberg et al. (162, 165) which could support the preferential incision (nicking) of UV-irradiated DNA relative to unirradiated DNA was an important milestone that was fundamental to this research endeavor. This process of specific DNA incision was shown to be dependent on the presence of divalent cations such as Mg^{2+} or Mn^{2+} and to have an absolute requirement for ATP. Consistent with the cellular biology and genetics of nucleotide excision repair, the specific nicking of UV-irradiated DNA was not observed with extracts of *uvrA*, *uvrB*, or *uvrC* mutant cells. However, extracts of these mutant cells could be complemented for nicking activity by addition of extract from a different *uvr* mutant strain (162, 163, 165) (Fig. 5–2).

Aside from the importance of appropriate preparation of cell extracts, it is now well recognized that the *uvrA*$^+$, *uvrB*$^+$ and *uvrC*$^+$ gene products are constitutively expressed in extremely small amounts in *E. coli*, and for many years attempts to purify these proteins were additionally frustrated by these quantitative limitations. The purification of the UvrA, UvrB, and UvrC proteins in high yields was enormously facilitated by the molecular cloning of the genes that encode them and by their extensive overexpression (63, 102, 142, 147, 152, 154, 170, 210, 226). The amplification of *uvr* gene expression can result in yields of Uvr proteins approaching 25 to 30% of the total soluble protein of *E. coli* cells, allowing the purification of milligram quantities of these proteins to physical homogeneity (205, 226, 232). As discussed in later chapters, the overexpression of recombinant nucleotide excision repair proteins from eukaryotic cells has been far less successful.

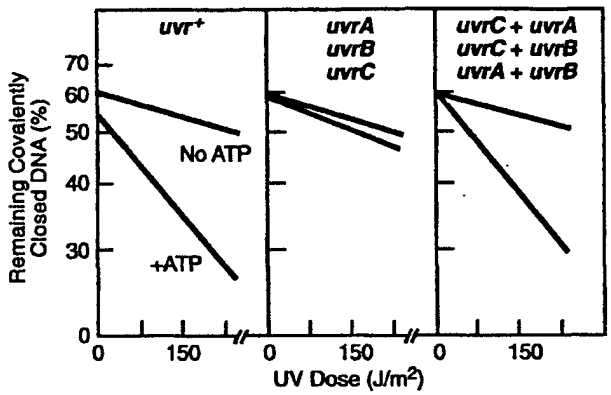


Figure 5–2 UV-irradiated DNA is incised by extracts of *E. coli* cells which are proficient for nucleotide excision repair (*uvr*$^+$) in the presence of ATP and Mg^{2+} (left panel). Extracts of *uvr* mutant cells fail to demonstrate the preferential incision of UV-irradiated DNA (center panel); however, mixtures of extracts from different *uvr* cells complement each other for nicking activity (right panel). Incision (nicking) of DNA was measured by the conversion of covalently closed circular (form I) DNA to the relaxed-circular or linear configurations. *(Adapted from Friedberg [45a] with permission.)*

The enzyme-catalyzed incision of damaged DNA during nucleotide excision repair in *E. coli* absolutely requires the UvrA, UvrB, and UvrC proteins. Regrettably, a standard nomenclature for this enzyme has not been developed. The enzyme has been variously referred to as the *UvrABC endonuclease*, the *UvrABC excinuclease*, the *Uvr(A)BC endonuclease*, and the *Uvr(A)BC excinuclease*. The term "excinuclease" was introduced in deference to the fundamental observation, discussed in detail below, that the enzyme catalyzes damage-specific incision of DNA and thereby facilitates the *excision* of an oligonucleotide generated during the incision reactions. The reason for denoting the UvrA protein in parentheses will become apparent later. In keeping with standard biochemical nomenclature, we prefer the term *endonuclease*, since the endonucleolytic incision of DNA is a primary biochemical event catalyzed by this enzyme. Hence, in this book we will use the name *UvrABC endonuclease*. Before discussing how these proteins effect damage-specific incision of DNA, let us consider the proteins themselves and the genes that encode them.

The *uvrA*$^+$ Gene and UvrA Protein

The *uvrA*$^+$ gene is located at 92 min on the *E. coli* genetic map. *uvrA* is one of a series of genes collectively referred to as *SOS genes*, which are induced to increased levels of transcription by agents that cause DNA damage. Details of the SOS response to DNA damage are discussed in chapter 10. For the purposes of the present discussion, it is relevant to note that the expression of SOS genes is regulated by a repressor which is the product of a gene called *lexA*$^+$. A specific binding site for the LexA repressor protein at the operator-promoter region of the *uvrA*$^+$ gene (the so-called *LexA box* or *SOS box*) has been identified (153). Not unexpectedly, similar sequences have been identified in the regulatory regions of other genes involved in the SOS response, including the *uvrB* gene (see below).

The *uvrA*$^+$ gene constitutes a single operon and is not cotranscribed with any other gene (170). The gene is separated by 253 bp from a gene called *ssb* (which encodes a single-stranded DNA-binding protein), but the two genes are transcribed divergently (170). The *uvrA* LexA binding site is located in this intergenic region and is required for inducible regulation of *uvrA* (13), but it is not involved in the expression of the *ssb* gene (170). There are only ~25 molecules of UvrA protein constitutively in *E. coli* cells, but SOS-mediated induction of the *uvrA* gene increases the intracellular content of UvrA protein to ~250 molecules per cell (170, 210).

Table 5–1 Properties of UvrA, UvrB, and UvrC proteins of *E. coli*

Property	Value for: UvrA	UvrB	UvrC
Mol mass (Da)	103,874	76,118	66,038
No. of amino acids	940	673	610
No. of Trp residues/molecule	3	0	2
Molar extinction coefficient	46,680	27,699	36,200
pI	6.5	5.0	7.3
Stokes radius (Å)	59	41	41
Sedimentation coefficient	7.4	4.2	4.9
Intrinsic metal	2 Zn	None	None
DNA binding	Yes	No	Yes
Nucleotide-binding motifs	2	1	None
ATPase activity	Yes	No	No
SOS regulation	Yes	Yes	No
No of molecules/cell	20 (250)[a]	250 (1,000)	10

[a] Numbers in parentheses indicate values after SOS induction.
Source: Adapted from Van Houten (210).

The cloned *uvrA*$^+$ gene contains an open reading frame of 940 amino acids, which can encode a polypeptide of about 103.9 kDa (78, 170, 210), in reasonable agreement with the molecular mass of the purified protein (~114 kDa) (Table 5–1) (205, 232). The protein has a Stokes radius of 59 Å (5.9 nm) (Table 5–1) and an S value of 7.4 (Table 5–1). It is a slightly acidic protein, with a measured pI of 6.5 (Table 5–1) (170).

UvrA protein is a DNA-independent ATPase and a DNA-binding protein. Both of these functional attributes correlate with specific structural motifs in the translated nucleotide sequence. The amino acid sequence reveals the presence of two consensus so-called Walker type A and type B purine nucleotide recognition motifs (223), found in many proteins which bind and hydrolyze ATP and/or GTP (152, 210). The type A sequences are the most highly conserved (Fig. 5–3). More detailed analysis of the predicted UvrA polypeptide and comparisons with the sequences of a number of other ATPases revealed regions of homology extending over approximately 250 amino acids, suggesting that UvrA protein is a member of a superfamily of prokaryotic ATPases, many of which are plasma membrane-bound proteins involved in active transport, multidrug resistance, cell division, nodulation during nitrogen fixation, protein export, recombination, and replication (40, 55, 71). The amino acid sequence of the UvrA protein also contains two zinc finger motifs (Fig. 5–3) (see below) and it has been suggested that a consensus

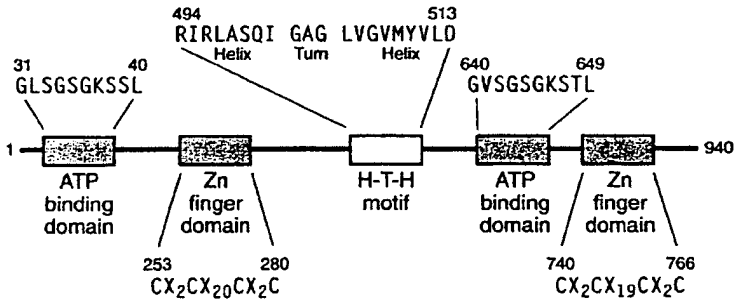


Figure 5–3 Diagrammatic representation of functional motifs identified in the amino acid sequence of the *E. coli* UvrA protein (940 amino acids). The location of the two Walker type A consensus sequences for nucleotide (ATP) binding are shown, as well as the two zinc finger domains and the helix-turn-helix (H-T-H) motif. (*Adapted from Grossman and Thiagalingam [62] with permission.*)

helix-turn-helix motif is present as well (Fig. 5–3) (224). Both the zinc finger and helix-turn-helix motifs are thought to be important determinants for the ability of the protein to bind to DNA. The C-terminal 44 amino acids of UvrA protein are rich in glycine residues (31) and are thought to be important for DNA damage recognition (see below).

Site-directed mutagenesis of UvrA protein suggests that it has two functional ATPase motifs located near the N-terminal and C-terminal regions of the polypeptide and corresponding to those identified by inspection of the amino acid sequence (203). The K_m for ATP of the wild-type protein is 149 μM. This value is intermediate between those independently calculated for the N-terminal site (60 μM) and for the C-terminal domain (312 μM). The apparent second order rate constant for the association of ATP with the C-terminal ATP-binding site is only slightly less than that of the full-length wild-type protein. However, the rate constant for association with the N-terminal site is about an order of magnitude lower. Hence, it would appear that the former site has a higher affinity for ATP than the latter has (203). The ATPase activity of UvrA protein has a k_{cat} of $\sim 1\ s^{-1}$ (205). However, there is a substantial decrease in the k_{cat} of the ATPase activity for each individual site compared with the native protein, suggesting that the two sites act cooperatively in ATP hydrolysis (203). The ATPase activity is stimulated ca. twofold by the simultaneous presence of unirradiated DNA and UvrB protein (but not by either alone) (205). The roles of ATP hydrolysis during the damage-specific incision of DNA by the UvrABC endonuclease are considered more specifically below.

When radioiodination of UvrA protein is carried out in the presence of ATP, significantly higher levels and initial rates of tyrosine iodination are observed than in the absence of ATP, suggesting that the binding of ATP to UvrA protein is associated with a conformational change in the protein (63, 226). In support of this suggestion, UvrA protein can exist as a monomer in the absence of ATP but dimerizes in its presence (152, 226). High concentrations of UvrA protein and the binding (but not the hydrolysis) of ATP favor dimerization of the protein (107), and the presence of the nonhydrolyzable ATP analog ATP[γS] also favors dimerization (107). At monomer-dimer equilibrium, the apparent association constant is $K_a = \sim 10^8\ M^{-1}$ (152). In the presence of ATP[γS] UvrA protein becomes trapped in its dimeric state, suggesting that ATP hydrolysis is linked to the reversal of UvrA dimerization (226). The exact stoichiometry of ATP binding to UvrA protein is $1ATP/(UvrA)_2$ (117). In summary, UvrA protein dimerizes in solution and the dimerization constant is increased by the binding of ATP. ATP binding (and possibly dimerization) results in a conformational change of the $(UvrA)_2$ protein complex.

The amino acid sequence domains that are involved in the binding and hydrolysis of ATP by Uvr A protein are separated by two zinc finger motifs with the prototypic sequence $CXXCX_{18-20}CXXC$ (12) (Fig. 5–3). These are believed to be involved in DNA-binding (152). Extended X-ray absorption fine-structure analysis of purified UvrA protein has shown that it does indeed contain two bound Zn atoms per molecule, each coordinated with four sulfur atoms (in cysteine residues) at a distance of 2.32 Å (0.23 nm) (121). The functional significance of these motifs is not certain. Substitution of Cys-253, the first cysteine residue in the N-terminal zinc finger (Fig. 5–3) by histidine, serine, or alanine results in a modest loss of the ability of the mutant gene to fully complement the UV radiation sensitivity of *uvrA* strains (121). On the other hand, independent studies suggest that the substitution of Cys-253 or Cys-256 (Fig. 5–3) with serine has little or no significant effect on the resistance of the mutant *uvrA* strains to UV radiation or on the activity of purified UvrA protein (219). However, substitution of Cys-763 in the C-terminal zinc finger motif (Fig. 5–3) confers extreme sensitivity to UV radiation and inactivates UvrA protein (219). Hence, it is possible that the second (C-terminal) zinc finger motif is required for protein binding to DNA, while the first (N-terminal motif) is required for the proper dimerization of UvrA protein.

Incomplete polypeptides comprising the 70-kDa N-terminal and 35-kDa C-

terminal regions of UvrA protein and carrying one or the other of these two zinc finger domains have been purified and characterized (117). When mixed, the two fragments fail to reconstitute functional UvrABC endonuclease activity in the presence of UvrB and UvrC proteins. Hence, it is likely that the specificity for binding to damaged DNA is provided by the proper orientation of the two zinc finger motifs relative to one another and is not an intrinsic property of the individual domains (117). A third zinc finger motif has also been identified in the UvrA protein, but it has diverged extensively, and its functional significance is unknown (152). Further evidence of a requirement for zinc for the functional integrity of UvrA protein derives from studies showing that when recombinant UvrA enzyme is expressed in an insoluble form, it can be solubilized by procedures that denature the protein and allow it to refold. Refolding requires zinc to reconstitute functional UvrA protein (30). As indicated above, in addition to the zinc finger motifs, UvrA protein has a putative consensus helix-turn-helix motif. Base substitution experiments with this motif suggest that it is required for the protein to recognize base damage in DNA (224).

The domains of the UvrA protein that are required for its various functional activities have been mapped by comparing the properties of various mutant polypeptides generated by systematic deletion mutagenesis (31). A region located in the N-terminal 230 amino acids is believed to be required for interaction with UvrB protein, whereas the region required for self-dimerization is within the first 680 amino acids. Almost the entire polypeptide (940 amino acids) is required for binding to DNA (31).

The binding of $(UvrA)_2$ protein to DNA has been demonstrated by a variety of experimental techniques (82, 107, 152, 205, 213, 219, 232). The protein binds nonspecifically to duplex DNA, with relatively low affinity. This nonspecific binding affinity is apparently higher for ends in duplex DNA and for single-stranded DNA (107, 152). In the absence of ATP, the protein binds more specifically to damaged DNA, binding to a psoralen adduct in duplex DNA with a binding constant $K_s = \sim 5 \times 10^7\ M^{-1}$ (152). The specificity of this binding increases another twofold in the presence of ATP hydrolysis. By using substrates containing the major products of UV radiation, it has been observed that the *off* (dissociation) rate for UvrA protein is exceedingly low in the absence of ATP hydrolysis but is stimulated in its presence (139). Thus, it appears that ATP hydrolysis increases the specificity of binding to damaged DNA but lowers the equilibrium binding constant by stimulating dissociation. DNA-binding affinity is also increased in the presence of ATP[γS] and is inhibited in the presence of ADP, suggesting that ATP hydrolysis is not required for this event (126, 166).

In its dimeric (and possibly ATP-bound) form, UvrA protein binds DNA containing various forms of base damage, ranging from AP sites to cross-link-initiated triple helixes (149, 170, 193, 215). However, these $(UvrA)_2$-DNA complexes are short lived and dissociate rapidly. Further selectivity for the binding of UvrA protein to damaged DNA is achieved by its specific interaction with UvrB protein. Before discussing this interaction, let us consider the second player in the triad of molecular components required for the damage-specific incision of DNA in *E. coli*, the *uvrB* gene and its polypeptide product.

The *uvrB*$^+$ Gene and UvrB Protein

The *uvrB*$^+$ gene maps at 17 min on the *E. coli* chromosome and hence is unlinked to *uvrA*. Like the *uvrA*$^+$ gene, *uvrB*$^+$ is monocistronic. Regulation of the *uvrB*$^+$ gene (which, as indicated above is also a member of the SOS regulon and is inducible by DNA damage) is complex (44, 158). Transcription of *uvrB*$^+$ appears to be controlled by both SOS-dependent and SOS-independent promoters (210). In vitro the gene is transcribed from two overlapping promoters called P_1 and P_2 (Fig. 5–4). A LexA protein binding site is present in the promoter region. In vitro this site apparently functions as the operator for P_2, since transcription from P_2 is inhibited in the presence of LexA repressor protein while that from P_1 is unaffected. On the other hand, there are indications that in vivo P_1 (the promoter closest to the coding region of *uvrB*) determines both constitutive and induced levels of

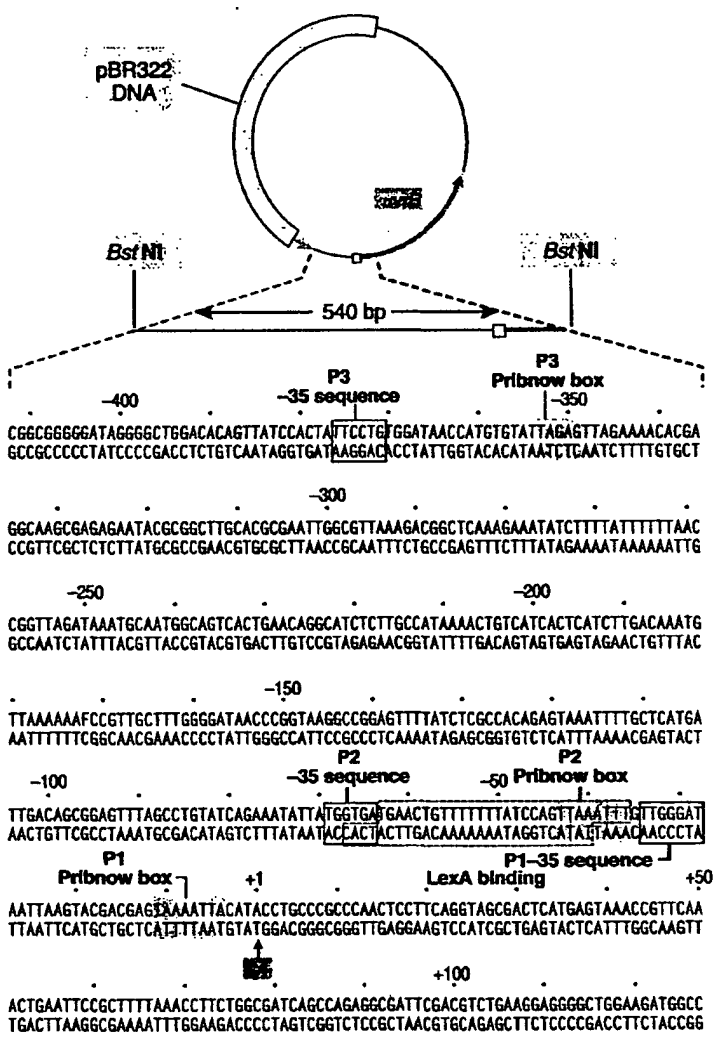


Figure 5–4 Nucleotide sequence of a 540-bp *Bst*NI fragment containing the *E. coli uvrB*$^+$ gene regulatory regions. The numbering of nucleotides is relative to a transcriptional start site designated S1. Pribnow boxes and −35 sequences for three promoters (P1, P2, and P3) are identified, as is a region protected from DNase I attack by the binding of LexA protein. (*Adapted from Friedberg [45a] with permission.*)

uvrB$^+$ transcription and that P_2 serves mainly to regulate transcription from P_1. In vitro the binding of LexA protein to P_2 may interfere with the binding of RNA polymerase to P_1 or, alternatively, may interfere with the local unwinding of DNA that precedes initiation of transcription (9, 208).

A third promoter, designated P_3, has been identified 320 bp upstream of P_2 (Fig. 5–4). In vitro, transcription from P_3 is directed toward the *uvrB* structural gene but terminates in the region of the LexA-binding site even in the absence of LexA protein (155). The physiological role (if any) of this promoter and the nature of its transcript are unknown. Plasmid vectors carrying the P_3 region can be propagated only in certain media or in strains deleted of *uvrB* (148, 155, 207). Mutations that modify a potential stem-loop structure in the P_3 region stabilize the propagation of plasmids (207). It has therefore been suggested that transcription from P_3 leads to plasmid loss and that the stem-loop structure acts as a regulatory

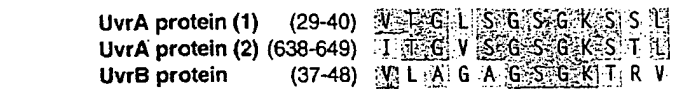


Figure 5–5 The Walker type A nucleotide binding motifs in the *E. coli* UvrA and UvrB proteins share extensive amino acid identity and similarity.

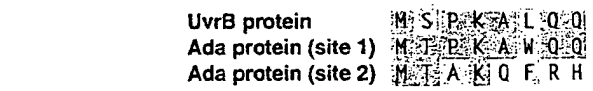


Figure 5–6 The *E. coli* UvrB protein has an amino acid sequence motif that resembles those observed in the *E. coli* Ada protein (see chapter 3). Those in the Ada protein are sites of preferred proteolytic cleavage of the protein.

site for this promoter (207). Interestingly, the putative stem-loop structure in P_3 shares sequence homology with the binding site for DnaA protein in the *E. coli* replication origin *oriC* (50), suggesting the interesting possibility that expression of the *uvrB*$^+$ gene is somehow coupled to DNA replication (207). In this regard, it may be relevant that *polA uvrB* mutants are inviable (111).

The 3′ untranslated region of the *uvrB*$^+$ gene contains so-called repetitive extragenic palindromic sequences (6, 8). Such sequences in other genes have been shown to be binding sites for DNA gyrase (229), suggesting that they may be involved in determining higher-order structure in the *E. coli* chromosome (210).

The *uvrB*$^+$ gene can encode a polypeptide of 673 amino acids with a calculated molecular mass of 76.6 kDa (6, 8). The amino acid sequence of UvrB shows several interesting features. First, like the UvrA amino acid sequence, the protein has a consensus Walker type A nucleotide-binding motif (Fig. 5–5). The purified protein has no detectable ATPase activity. However, as discussed below, there are indications that UvrB may be a cryptic ATPase which becomes activated only when bound to UvrA protein or when degraded by specific proteolysis (23).

A second notable feature of UvrB protein is the presence of an amino acid sequence motif which is very similar to that known to function as a specific proteolytic cleavage site in the *E. coli* Ada protein (DNA alkyltransferase I) (Fig. 5–6) (see chapter 3) (6, 202). Indeed, a proteolysis product of UvrB protein designated UvrB* has been identified in extracts of *E. coli*. The suggested physiological significance of this degradation reaction is discussed below. Finally, the amino acid sequence of the UvrB protein shows homology with two limited stretches of the sequence of the UvrC protein (210). The more extensive of these is a stretch of 14 amino acids located close to the C terminus of the UvrB polypeptide, 13 of which are identical to a region in the middle of the UvrC polypeptide (Table 5–2). As discussed below, a UvrC polypeptide that contains just the C-terminal 314 amino acids and excludes this region of homology with UvrB protein is sufficient to reconstitute functional UvrABC activity in vitro (100). However, the resistance to UV radiation of mutant strains carrying this deleted UvrC protein is considerably reduced, although it is not clear that this reduced survival specifically reflects a requirement for the UvrB-like motif in UvrC protein.

Purified UvrB protein has a relative molecular mass of 84 kDa (Table 5–1), slightly larger than that predicted from the open reading frame of the *uvrB* gene

Table 5–2 Homology between the UvrB and UvrC polypeptides

Polypeptide (nt)[a]	Sequence
UvrB (649–664)	AQNLGFEEAAQINDQL
UvrC (198–213)	SQNLGFEEAANINDQI

[a] nt, nucleotides.

(148, 205, 230). It is a globular acidic protein with a Stokes radius of 41 Å (4.1 nm), an S value of 5.2, and a pI of 5.0 (Table 5–1) (170). The protein is monomeric in solution and does not bind ATP or DNA in isolation (81, 213). However, UvrB protein interacts specifically with UvrA protein to form protein-protein and protein-DNA complexes which are important intermediates in the biochemistry of the damage-specific incision of DNA. UvrB protein is constitutively expressed at a level of ~250 molecules per cell (significantly higher than that of UvrA protein [see above]). Following SOS induction, the level of UvrB protein increases to as much as ~1,000 molecules per cell (170).

INTERACTIONS BETWEEN UvrA AND UvrB PROTEINS—THE PHENOMENON OF DAMAGE-SPECIFIC RECOGNITION

As indicated above, a fundamental aspect of the specificity of nucleotide excision repair is the ability of the repair machinery to locate and identify base damage in the genome, a process referred to as *damage-specific recognition*. In *E. coli*, this recognition problem is apparently solved by interactions between a complex of the UvrA and UvrB proteins and their interactions with damaged DNA.

Purified UvrA protein associates with UvrB protein to form a $(UvrA)_2(UvrB)_1$ complex (Fig. 5–7). At physiological concentrations (~10 nM UvrA protein and ~200 nM UvrB protein), all UvrA protein is in the form of $(UvrA)_2(UvrB)_1$ complexes (130). This interaction is strictly dependent on the presence of ATP (130). ATP[γS] cannot be substituted for ATP in this binding reaction, suggesting that ATP hydrolysis rather than ATP binding is critical for $(UvrA)_2(UvrB)_1$ complex

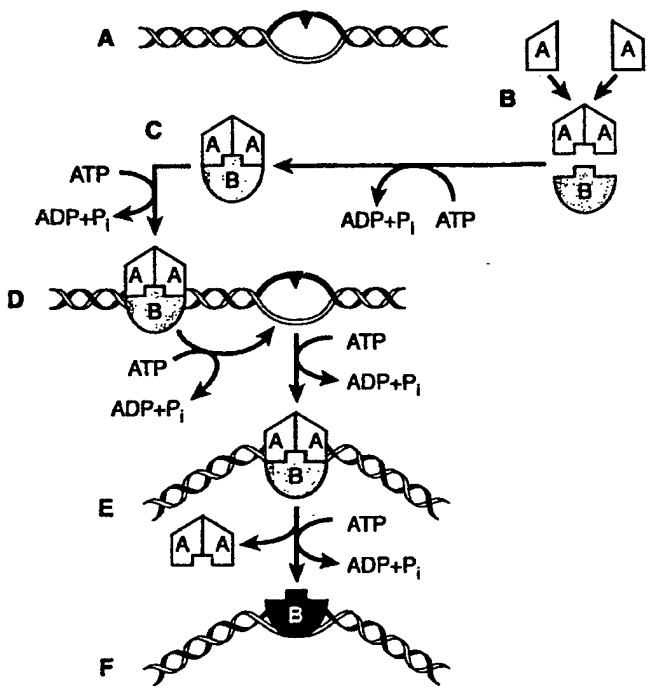


Figure 5–7 Model for the formation of a stable $(UvrB)_1$–damaged-DNA complex during nucleotide excision repair in *E. coli*. A site of base damage on one DNA strand is represented diagrammatically (A). A $(UvrA)_2(UvrB)_1$ protein complex forms in solution (B and C) and initially binds to DNA at a site removed from the damage (D). Promoters in genes might constitute preferential sites for the initial "docking" of such complexes. The $(UvrA)_2(UvrB)_1$ complex tracks along the DNA using a DNA helicase activity (D). When the site of base damage is encountered (E), UvrA protein dissociates from the complex, leaving a stable $(UvrB)_1$-DNA complex (F). This is associated with bending and kinking of the DNA.

formation (Fig. 5–7) (152). $(UvrA)_2(UvrB)_1$ complexes have a higher binding affinity for damaged DNA than does UvrA alone. In contrast, there are indications that the reverse is true for undamaged DNA (211). As indicated previously, the association constant for the specific binding of UvrA protein to a psoralen adduct in duplex DNA is $\sim 5 \times 10^7\ M^{-1}$ in the absence of ATP and UvrB protein (152). In the presence of these factors it increases to $\sim 1 \times 10^9\ M^{-1}$ (152), resulting in a significantly increased affinity for damaged relative to undamaged DNA.

It has been suggested that the fundamental role of UvrA protein in nucleotide excision repair is to function as a "molecular matchmaker" which delivers UvrB protein to sites of distortive damage in DNA by the formation of a transient $(UvrA)_2(UvrB)_1$-DNA complex from which it rapidly dissociates, leaving a highly stable $UvrB_1$-DNA complex (Fig. 5–7) (102, 130, 150, 154). The concept of molecular matchmaking embodies the notion that UvrA protein serves as a transient component of a multiprotein complex and does not itself directly participate in biochemical events after the stable binding of UvrB protein at sites of base damage. It is for this reason that the UvrA protein is designated parenthetically in the term Uvr(A)BC endonuclease. This concept is not without precedent. Molecular matchmakers have been defined as a class of proteins which use the energy of ATP hydrolysis to effect conformational changes in one or both components of a *binding protein–DNA target pair*. They are believed to participate in key protein-DNA interactions in several distinct aspects of DNA metabolism, including replication, transcription, mismatch repair, and possibly recombination and transposition; it has been hypothesized that they provide a fundamental mechanism for high-avidity binding to DNA by proteins which cannot rely on sequence specificity for such interactions (130, 150).

This model derives from several observations with respect to the UvrA-UvrB interaction. First, in the presence of UvrB protein, the transient 33-bp $(UvrA)_2$ footprint observed at a psoralen monoadduct is reduced to a stable smaller (19-bp) footprint thought to represent the binding of UvrB alone to DNA (14, 152, 213). Second, when UvrA and UvrB protein are incubated in different proportions in the presence of UV-irradiated DNA and DNA-protein complexes are isolated and analyzed for protein content, the ratio of UvrA to UvrB (as bound protein) decreases as the amount of UvrA protein in the reaction mixtures is reduced, and it eventually becomes vanishingly small, suggesting that only UvrB protein is bound to the irradiated DNA. Under conditions of saturating UvrB protein, one UvrB molecule is bound per damaged site in a reaction that requires only catalytic amounts of UvrA protein (130).

While there is a consensus among several laboratories that the $(UvrA)_2(UvrB)_1$ complex participates in the process of damage-specific recognition, there is less consensus about exactly how this process transpires and the precise events by which a stable $(UvrB)_1$-DNA preincision complex ultimately assembles at sites of base damage. Using gel shift analysis with a 49-bp duplex oligonucleotide containing a benzoxyamine-modified AP site, it has been observed that the interaction of the $(UvrA)_2(UvrB_1)$ protein complex with a benzoxyamine site results in three detectable intermediates, consistent with the presence of a $Uvr(A)_2$-DNA complex, a $(UvrA)_2(UvrB)_1$-DNA complex, and a $(UvrB)_1$-DNA complex (215). Similar results have been obtained with a 96-bp oligonucleotide substrate containing a cisplatin adduct in a defined position (218). The $(UvrA)_2$ complex bound to DNA containing a benzoxyamine site is short-lived, with a half life of ~15 s. However, the other two complexes, which include UvrB protein, have half lives of ~2 h and appear to be in equilibrium (215). The relative amount of each is suggested to be dependent on the concentrations of UvrA and UvrB proteins, the nature and the amount of the damaged DNA, and the DNA concentration (215). These variables may contribute to the different efficiencies of incision by the UvrABC endonuclease observed for different types of DNA damage (see below).

The delivery of UvrB protein to sites of damage in UV-irradiated DNA has an absolute requirement for both UvrA protein and ATP hydrolysis (131). Effective loading of UvrB protein occurs in a limited range of UvrA protein concentration

(5 to 50 nM) and is inhibited at high concentrations. The rate of loading of UvrB protein is low ($k_{on} = 6 \times 10^{-4}$ M/s), suggesting that this step may be limiting for damage-specific incision of DNA. The K_m of ATP hydrolysis during the loading of UvrB protein is very similar to that for ATP hydrolysis by UvrA protein alone, suggesting that ATP hydrolysis during this loading reaction is effected by UvrA protein (131). Consistent with this interpretation, substitution of the Lys residue with Ala in the invariant tripeptide GKS/T of the most N-terminal and C-terminal Walker type A boxes inactivates the ability of UvrA protein to hydrolyze ATP. These amino acid substitutions have a marginal effect on the ability of UvrA protein to bind to damaged DNA, but they drastically reduce the ability of the protein to deliver UvrB protein to sites of base damage (117).

Transient $(UvrA)_2(UvrB)_1$-DNA complexes have not been unambiguously detected, nor has the binding constant for $(UvrB)_1$-DNA been measured. However, isolated $(UvrB)_1$-damaged DNA complexes are very stable, with a k_{off} of 1.1×10^{-4} s^{-1}, which extrapolates to a half-life of 2 to 3 h, depending on the particular type of base damage (130). Once a relatively stable $(UvrA)_2(UvrB)_1$-DNA complex has formed, ATP hydrolysis by the activated UvrB ATPase function is thought to be accompanied by kinking and local denaturation of the DNA and a concomitant change in the conformation of UvrB protein. These changes are believed to result in the tight binding of UvrB protein to sites of base damage and to the release of UvrA protein (Fig. 5–7) (154). Indeed, experiments that have exploited the ability of dinuclear platinum compounds to both damage DNA and cross-link proteins have resulted in the identification of UvrB-platinum-DNA ternary complexes (214). Hence, while DNA damage recognition by UvrA protein is a process of relatively low specificity, extremely high specificity is attained by the UvrA-dependent binding of UvrB protein at sites of base damage. $(UvrB)_1$-DNA complexes have not been detected with undamaged DNA (170).

Experimental evidence in support of conformational alterations associated with the binding of UvrB protein to damaged DNA comes from various sources (97, 226). Measurements of DNA unwinding in the presence of UvrA and UvrB proteins and ATP suggest the unwinding of as much as one full helical turn per Uvr protein-DNA complex (126, 226). This unwinding might be a direct effect of the binding of the Uvr proteins to DNA. There are certainly ample precedents in the literature for this. For example, during the formation of a stable protein-DNA complex, *E. coli* RNA polymerase has been shown to unwind the DNA helix by ~17 bp. Similarly, catabolite gene activator protein and *Eco*RI endonuclease have been shown to unwind the helix during binding to specific cognate sequences (210). Alternatively, the Uvr proteins could unwind DNA via a DNA helicase activity. Evidence for such an activity as an intrinsic property of a UvrA-UvrB complex is discussed below. In addition to evidence of local denaturation of DNA, electron microscopy indicates that after formation of the $(UvrA)_2(UvrB)_1$-DNA ternary complex the DNA is kinked by ~130° (150, 180) (Color Plate 4). Further evidence in support of alterations in the structure of DNA upon its interaction with UvrB protein derives from flow linear dichroism studies (198).

The UvrB footprint at sites of psoralen monoadducts in DNA is most pronounced on the undamaged strand, suggesting that UvrB protein makes more intimate contact with that strand. In support of this conclusion, it has been observed that *E. coli* DNA photolyase (which binds to the damaged strand at pyrimidine dimer sites [see chapter 3]) does not inhibit damage-specific incision of UV-irradiated DNA in the presence of the *E. coli* Uvr proteins; in fact, it stimulates the incision reaction (76, 149). Thus, the endonuclease apparently binds at the DNA face opposite to the one containing the adduct. The only distinctive effect on the damaged strand is the appearance of a DNase-hypersensitive phosphodiester bond (170).

What specific determinants guide the precise interactions of the $(UvrA)_2(UvrB)_1$ protein complex with damaged DNA? One possibility is that $(UvrA)_2(UvrB)_1$ protein complexes randomly patrol the genome and that specific binding is guided by the affinity of UvrA for particular types of DNA distortions.

The more "correct" (i.e., distinctive from native DNA) the distortion, the greater is the probability of productive $(UvrA)_2(UvrB)_1$-DNA complex formation, i.e., one that leads to the dissociation of UvrA protein and the formation of a stable $(UvrB)_1$-DNA complex. The formation and stability of productive intermediates may also be influenced by the extent to which the DNA distortion initially recognized is progressively modified during the course of these reactions. Stated differently, it has been suggested (62, 226) that the DNA substrate may be progressively determined by conformations imposed on it as the result of its interactions with the UvrA-UvrB protein complex. Hence, the initial conformational distortion in damaged DNA may simply serve to lower the K_m for the binding of $(UvrA)_2(UvrB)_1$ complexes (62, 226). This concept predicts a distinctive hierarchy for the kinetics of damage-specific incision of DNA substrates with different types of helix-distortive damage. Experiments with different types of base damage suggest that this is indeed the case (226).

As indicated above, the difference in the affinity of UvrA protein for damaged and undamaged double-stranded DNA is low (a factor of only 10^3 to 10^4). Statistical considerations indicate that under conditions where only a single nucleotide in 10^7 (or fewer) is damaged, initial Uvr protein-DNA interactions will more probably occur at undamaged than at damaged sites in DNA (64). These considerations have prompted consideration of a mechanism by which the initial formation of a nonspecific $(UvrA)_2(UvrB)_1$-DNA complex is followed by translocation of the Uvr proteins to sites of distortive damage, yielding specific $(UvrA)_2(UvrB)_1$-DNA complexes (58, 60, 61, 63, 64).

It has in fact been shown that a UvrA-UvrB complex [presumably the $(UvrA)_2(UvrB)_1$ complex discussed above] is capable of limited DNA helicase activity in the presence of ATP (127, 128). This helicase can unidirectionally unwind very short stretches of DNA duplexes and DNA D-loop structures with strict 5′ → 3′ polarity, and it is inhibited by the presence of bulky base damage in UV irradiated DNA. While a DNA helicase could potentially function in several roles during nucleotide excision repair, the properties described above are consistent with a role in the translocation of a $(UvrA)_2(UvrB)_1$ complex from a site of relatively weak-affinity binding in DNA to the actual site of base damage, where the inhibitory effect of a bulky adduct might arrest the complex. Consistent with this model, mutational alteration of the Lys-45 residue located in the Walker type A nucleotide binding motif of the UvrB polypeptide leads to defective ATPase and DNA helicase activity in UvrA-UvrB complexes and a failure to support the incision of UV-irradiated DNA (167).

The ATPase that presumably drives the DNA helicase function of the UvrA-UvrB complex is believed to be cryptic in UvrB protein (6, 205) but becomes activated when it undergoes a conformational change associated with binding to UvrA protein and DNA (23). In keeping with this notion of a cryptic ATPase activity, UvrB* protein generated by proteolysis of UvrB protein in vivo possesses a DNA-dependent ATPase activity in the absence of UvrA protein (23).

An interesting question is whether the $(UvrA)_2(UvrB)_1$ complex has preferred sites of docking with DNA. By footprinting analysis it has been observed that the complex binds preferentially to the nontranscribed strand of promoter sites downstream from the RNA polymerase-promoter complex. This strand preference is consistent with the 5′ → 3′ directionality of the UvrAB helicase activity (3) and suggests that the binding of RNA polymerase to promoter sites in DNA may provide a signal for selective landing sites for the $(UvrA)_2(UvrB)_1$ complex on DNA (3).

In summary, a reasonable working model (Fig. 5–7) which is consistent with the consensus experimental evidence is that the binding of dimeric UvrA protein to UvrB protein in solution leads to the assembly of a $(UvrA)_2(UvrB)_1$ complex. This complex binds to DNA nonspecifically but is able to unwind DNA by using the energy of ATP hydrolysis (possibly by the bound UvrB protein), and it translocates unidirectionally (62). This may represent a fundamental DNA patrolling mechanism whereby the integrity of the genome is constantly monitored for base

damage in living cells. Such a patrolling mechanism is not limited to a three-dimensional diffusion process in solution but utilizes a mechanism which effectively decreases the volume of solution that must be "searched" by proteins while in a nonspecifically bound state (221). It has been suggested that when a site of base damage is encountered the $(UvrA)_2(UvrB)_1$ complex unwinds and kinks the DNA in a particular manner, UvrA protein dissociates, and a stable $(UvrB)_1$-DNA complex is formed in which the DNA is now conformationally primed for incision (102, 150, 210).

It remains to be determined precisely how sites of base damage facilitate the formation of stable $(UvrB)_1$-DNA complexes once such sites have been recognized and how these sites are distinguished from temporary changes in the local dynamics of undamaged DNA. This question is particularly pertinent when we consider the extremely broad range of substrates that are recognized by the UvrABC system in vitro (see below). An intriguing possibility is that the $(UvrA)_2(UvrB)_1$ complex recognizes lesion-imposed restrictions of the normal dynamic range of DNA (138). In other words, the complex may systematically scan the DNA for normal helical parameters by constantly inducing limited conformational changes in structure and testing these. Registration ("sensing") of normal binding interactions is followed by dissociation of the complex. However, any lesion which constrains some subset of conformations available to native DNA results in "jamming" of the protein-DNA complex in a high-affinity conformation (138). Any structural modification of DNA that causes the $(UvrA)_2(UvrB)_1$ complex to unload UvrB protein onto DNA is recognized as a substrate for subsequent endonucleolytic incision (98). The attractive feature of this type of model is the suggestion that it requires alterations in the *dynamics* of the DNA helix imposed by base damage, rather than alterations in its shape or *static* structure to determine substrate recognition (98, 138). It has been suggested that a particular aspect of the dynamic state of DNA that may determine the formation of stable $(UvrB)_1$-DNA complexes in the presence of multiple diverse types of base damage is an alteration in the energy of base stacking interactions in DNA (215). UvrB protein (which is quite hydrophobic) may "read" destabilized base stacking interactions in the damaged strand. In this regard it may be relevant that UvrB protein and single-stranded DNA-binding protein share limited regions of amino acid sequence homology (215).

The *uvrC*$^+$ Gene and UvrC Protein

The final step in the assembly of the catalytically active damage-specific endonuclease in *E. coli* is the binding of UvrC protein to specific $(UvrB)_1$–damaged-DNA complexes (Fig. 5–8). UvrC protein is the product of the *uvrC*$^+$ gene, which maps at 42 min on the *E. coli* genetic map. *uvrC*$^+$ is one of two genes in a single operon. The more proximal gene encodes a protein of 23 kDa. It has been pointed out that the amino acid sequence of this 23-kDa protein is homologous with several known prokaryotic positive regulators of gene expression (122, 170). However, the protein is apparently not involved in the regulation of *uvrC*, since its inactivation has no effect on the sensitivity of *E. coli* cells to UV radiation (110).

In addition to its cotranscription with the gene for the 23-kDa protein, *uvrC*$^+$ is monocistronically transcribed from an internal promoter (54, 179). Like the *uvrA*$^+$ and *uvrB*$^+$ genes, *uvrC*$^+$ is weakly expressed constitutively and there are only 10 to 20 molecules of the protein per cell (170). Unlike the other two *uvr* genes, however, *uvrC* is not inducible by DNA damage and is not a member of the SOS regulon (45, 56, 156).

The *uvrC*$^+$ gene is expected to encode a polypeptide of 610 amino acids with a calculated size of ~68.5 kDa (Table 5–1) (99). The amino acid sequence shows no remarkable features; nucleotide binding domains and zinc fingers are not present in the UvrC polypeptide (170). Purified UvrC protein is essentially neutral, with a pI of 7.3 (Table 5–1). It is a globular protein with a Stokes radius of 41 Å (4.1 nm) and an S value of 4.9 (Table 5–1) (170). The purified protein has a strong tendency to aggregate and becomes inactivated on prolonged storage (204).

UvrC protein does not associate with either UvrA or UvrB protein in solution, but it has a high affinity for the $(UvrB)_1$-DNA complex. The stoichiometry of UvrC

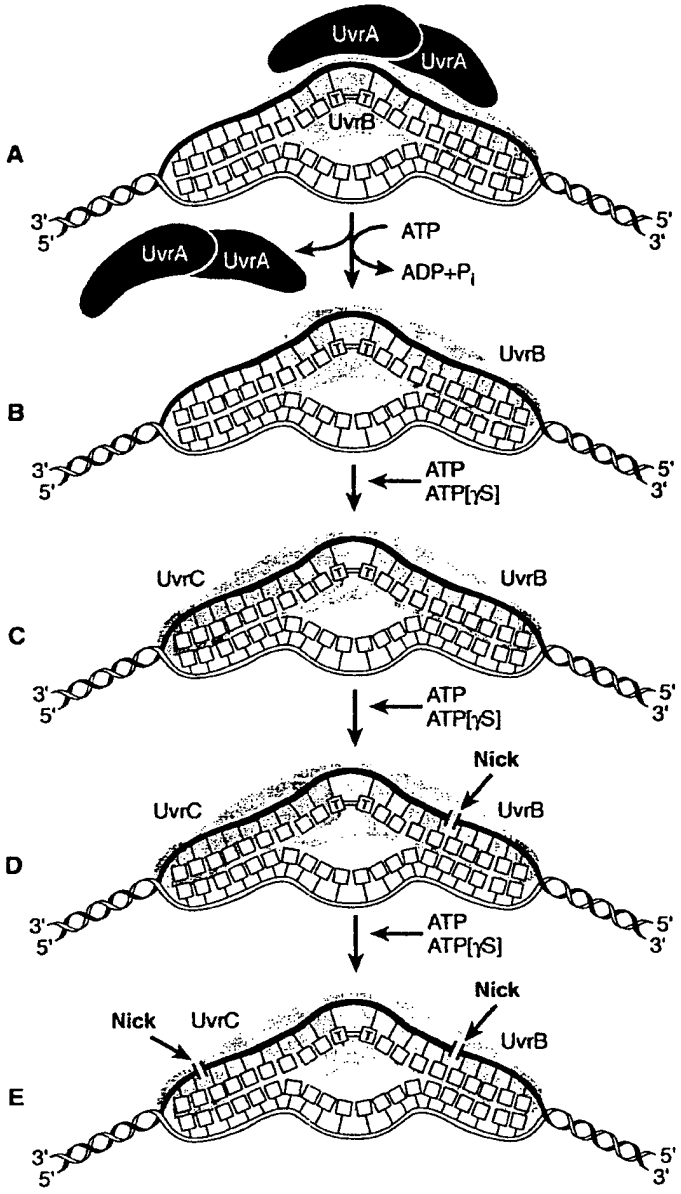


Figure 5–8 Diagrammatic representation of bimodal damage-specific nicking of DNA by the *E. coli* UvrABC endonuclease. Following the formation of a stable $(UvrB)_2$-damaged-DNA complex (A and B) (see Fig. 5–7), UvrC protein binds at the site (C) and induces a conformational change which enables bound UvrB protein to nick the DNA 4 nucleotides 3′ to the site of damage (D) (shown as a pyrimidine dimer). This reaction requires the binding of ATP (or ATP[γS]) by UvrB protein, but no ATP hydrolysis occurs at this step. Following the 3′ incision, UvrC protein catalyzes nicking of the DNA 7 nucleotides 5′ to the dimer (E).

binding in this reaction is uncertain. When the UvrA, UvrB, and UvrC proteins are incubated with damaged DNA in the presence of ATP, specific incision of the DNA results. Incision can also be effected by the addition of purified UvrC protein to UV-irradiated DNA carrying a single molecule of UvrB protein specifically bound at each site of base damage (130). This incision reaction is relatively slow; it takes about 2 min to complete in the presence of a vast molar excess of UvrC protein, a curious observation since in vivo UvrC protein is present in very low abundance (Table 5–1). ATP is required for the incision of damaged DNA when UvrC protein is added to the $(UvrB)_2$-DNA complex (Fig. 5–8), but there is no ATP hydrolysis. Indeed, the requirement for a nucleotide cofactor in the incision reaction can be supplanted by nonhydrolyzable analogs of ATP, suggesting that nu-

cleotide binding causes a conformational change which is required for damage-specific incision (131).

DNA INCISION IS BIMODAL

For a disquieting number of years (in retrospect), molecular models of nucleotide excision repair in *E. coli* invoked one strand break exclusively on the 5′ side of the sites of base damage. The chief imperative for this model was the known propensity for *E. coli* DNA polymerase I to degrade DNA at nicked sites in the 5′ → 3′ direction by a process called *nick translation*. Hence, it was intuitively persuasive that the combined actions of a 5′ DNA damage-specific endonuclease, the 5′ → 3′ exonuclease of DNA polymerase I, and DNA polymerase I-catalyzed repair synthesis, could account fully for the mechanism of nucleotide excision repair of damaged DNA, and this biochemical issue was considered essentially solved. However, when DNA sequencing gels were used to define the precise location of UvrABC protein-dependent incisions relative to pyrimidine dimers in DNA, the surprising and unexpected observation was first made by Sancar, Rupp, and coworkers (146, 151) that the damaged strand contained nicks on both sides of each dimer (Fig. 5–8), an observation confirmed by others (230, 231). Historically it is now amusing to recall that these investigators were as surprised by this result as the rest of the scientific community. Wedded to the 5′ incision model in vogue at the time, they sequenced the damaged DNA strand labeled at one end. Sequencing of the same strand carrying a radiolabel at the other end was carried out to confirm the location of the presumed single incision but, instead, led to the seminal observation that there were in fact two nicks flanking each dimer site. As discussed in later chapters, bimodal incision is apparently universal in nature during nucleotide excision repair.

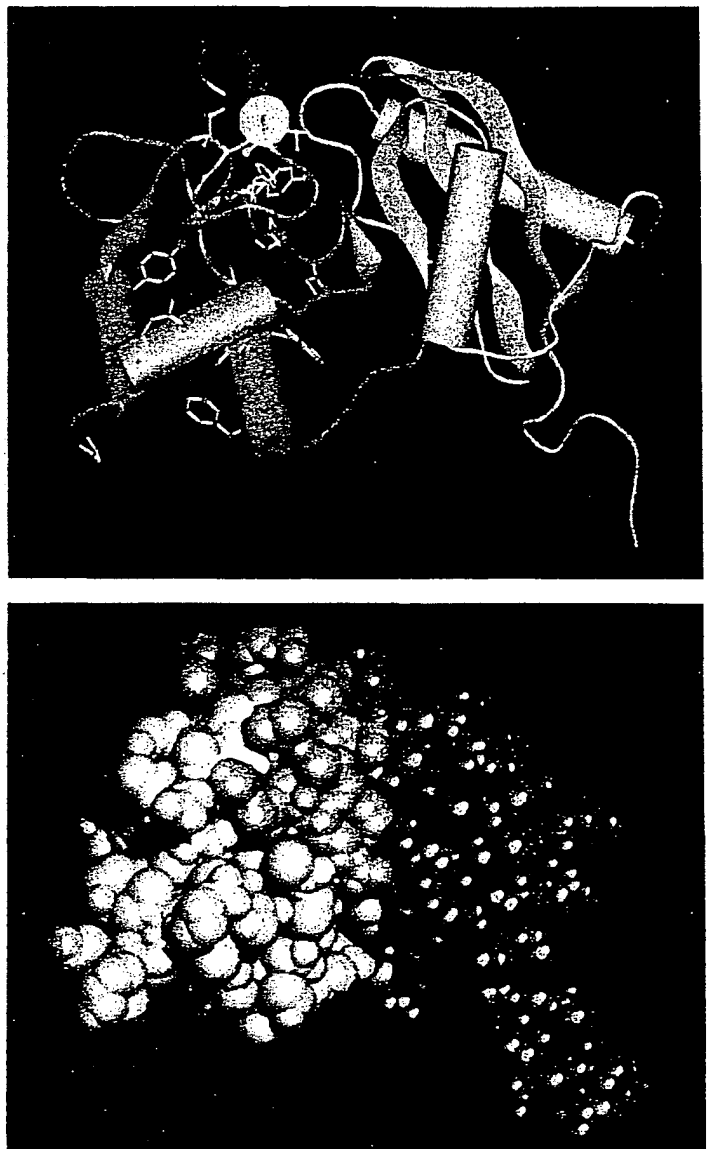
The complete UvrC polypeptide is not required for this incision reaction. The presence of the C-terminal 314 amino acids is sufficient to confer resistance to killing by UV radiation in *uvrC* mutants (100) (Fig. 5–9). Additionally, when the portion of the *uvrC* gene that encodes this region is fused to a gene that encodes maltose-binding protein, the fusion protein results in efficient nicking of UV-irradiated DNA in the presence of the UvrA and UvrB proteins (100).

The UvrABC damage-specific endonuclease typically hydrolyzes the eighth phosphodiester bond 5′ to pyrimidine dimers or (6-4) photoproducts in UV-irradiated DNA (6, 130) (Fig. 5–8 and Color Plate 5). The sites of incision 3′ to these lesions are more variable. Incision occurs at the fifth phosphodiester bond 3′ to all pyrimidine dimers (Fig. 5–8) and at the fourth phosphodiester bond 3′ to (6-4) photoproducts (151, 195, 230). In DNA containing cisplatin diadducts the 3′ incision site is almost exclusively at the fourth phosphodiester bond (10). DNA containing either psoralen monoadducts (either pyrone or furone), *N*-acetoxyacetylaminofluorene, cyclohexylcarbodiimide, 4-nitroquinoline-1-oxide, or mitomycin monoadducts is cut at the fifth 3′ phosphodiester bond.

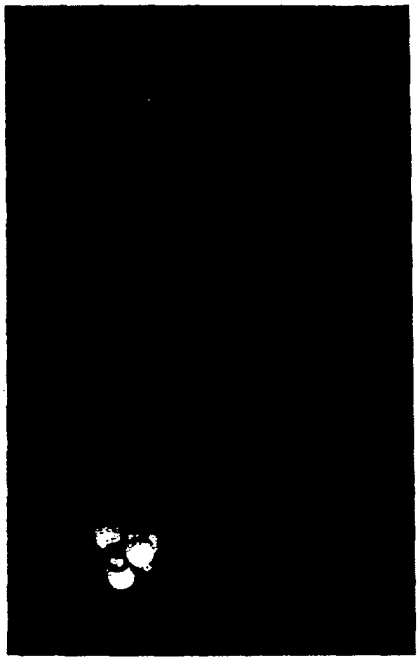
The precise locations of both 5′ and 3′ incisions can also be affected by DNA sequence context. For example, with both (6-4) photoproducts and pyrimidine dimers, incisions have been observed as close as the sixth bond on the 5′ side of lesions and as removed as the sixth phosphodiester bond on the 3′ side depending on the particular DNA sequence context in which these lesions are located (118). Additionally, when DNA fragments were constructed in which acetylaminofluorene adducts were specifically introduced at each of three different guanine residues in the sequence -GGCGCC-, major differences were detected in the efficiency of DNA incision following incubation with the UvrABC endonuclease (164). In contrast, all three adducts were recognized with equal efficiency as judged by DNase I protection experiments (164).

Site-specific mutagenesis of the UvrC and UvrB proteins has led to the important conclusion that the incision process is not a concerted one. The 3′ incisions are apparently effected by UvrB protein, and this incision precedes the 5′ cut catalyzed by UvrC protein (Fig. 5–8) (97, 101). Hence, in effect there are two physically distinct endonuclease activities in the *E. coli* UvrABC enzyme. This, too, might turn out to be universal. As will be seen in the next chapter, two endonu-

Color Plate 1 Space-filling model of an oligonucleotide containing a thymine dimer, showing proposed contact sites for *E. coli* DNA photolyase. A photolyase footprint generated by digestion with methidium propyl-EDTA-Fe(II) is shown in the cyan color. The red color indicates phosphates and N-7 atoms of guanine that are implicated in contacting the enzyme. The yellow color represents the cyclobutane ring of the pyrimidine dimer. (Reproduced with permission from I. Husain, G. B. Sancar, S. R. Holbrook, and A. Sancar. 1987. Mechanism of damage recognition by *Escherichia coli* DNA photolyase. *J. Biol. Chem.* **262**: 13188–13197. Photograph courtesy of A. Sancar.)

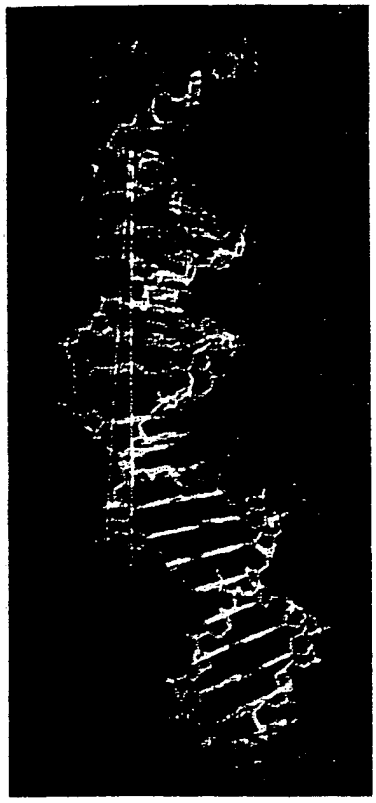


Color Plate 2 *(Top)* Schematic stereo representation of the 178-amino-acid C-terminal fragment of the *E. coli* Ada protein. The secondary-structure elements (α-helices and random coils) of the N-terminal region of this fragment are shown in blue, and of the C-terminal regions are shown in red and green. The side chains of the amino acid residues in the C-terminal domains shown as ball and stick are conserved in the sequences of 10 different O^6-alkyltransferase proteins from several prokaryotes and eukaryotes. *(Bottom)* Space-filling model of the structure shown in the top part of the plate using the identical orientation of display and the same color coding. The yellow speck represents the side chain of the active-site cysteine Cys-321 in the native protein, which is buried in the center of the fragment. (Reproduced with permission from M. H. Moore, J. M. Gulbis, E. J. Dodson, B. Demple, and P. C. E. Moody. 1994. Crystal structure of a suicidal DNA repair protein: the ada O^6-methylguanine-DNA methyltransferase from *E. coli*. *EMBO J.* 13:1495–1501. Photograph courtesy of M. Moore.).



Color Plate 3 Ribbon representation of the proposed structure of the *E. coli* Nth protein (endonuclease III) showing the bilobal organization of the elongated polypeptide separated by a deep cleft. The amino acid residues shown in red are identical in the Nth and homologous MutY proteins (see Fig. 4–15). Green residues are conserved in the two proteins, while blue residues are not homologous. Endonuclease III contains an iron (shown in brown)-sulfur (shown in yellow) cluster, which is bound entirely within a C-terminal loop. (Photograph courtesy of R. Cunningham.)

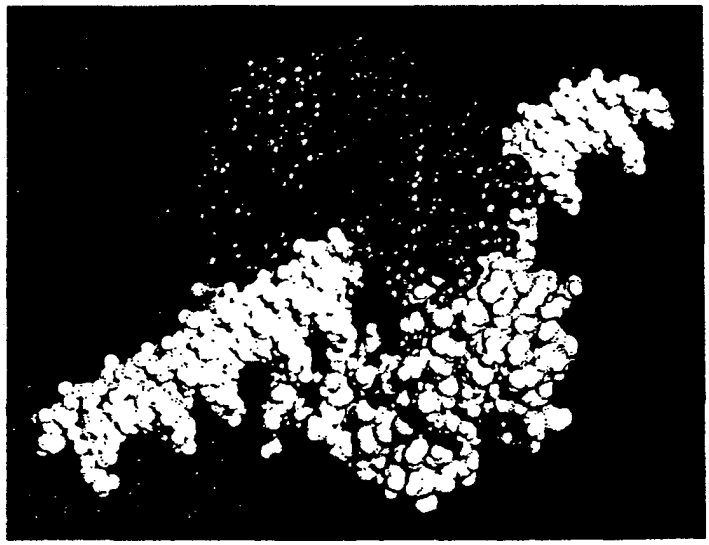
Color Plate 4 Electron micrograph showing kinking of DNA molecules at the point of UvrB protein binding. DNA bound to wild-type protein is severely kinked (left). A mutant form of UvrB protein in which the amino acid residue Asp-478 was replaced by Ala eliminates the bending of DNA while still allowing loading of the protein by UvrA protein at a site of base damage (right). (Reproduced with permission from D. S. Hsu, M. Takahashi, E. Delagoutte, E. Bertrand-Burggraf, Y. H. Wang, B. Norden, R. P. P. Fuchs, J. Griffith, and A. Sancar. 1994. Flow linear dichroism and electron microscopic analysis of protein-DNA complexes of a mutant UvrB protein which binds to but cannot kink DNA. *J. Mol. Biol.* **241**:645–650, and Q. Shi, R. Thresher, A. Sancar, and J. Griffith. 1992. Electron microscopic study of (A)BC excinuclease. DNA is sharply bent in the UvrB-DNA complex. *J. Mol. Biol.* **226**:425–432. Photograph courtesy of A. Sancar.).



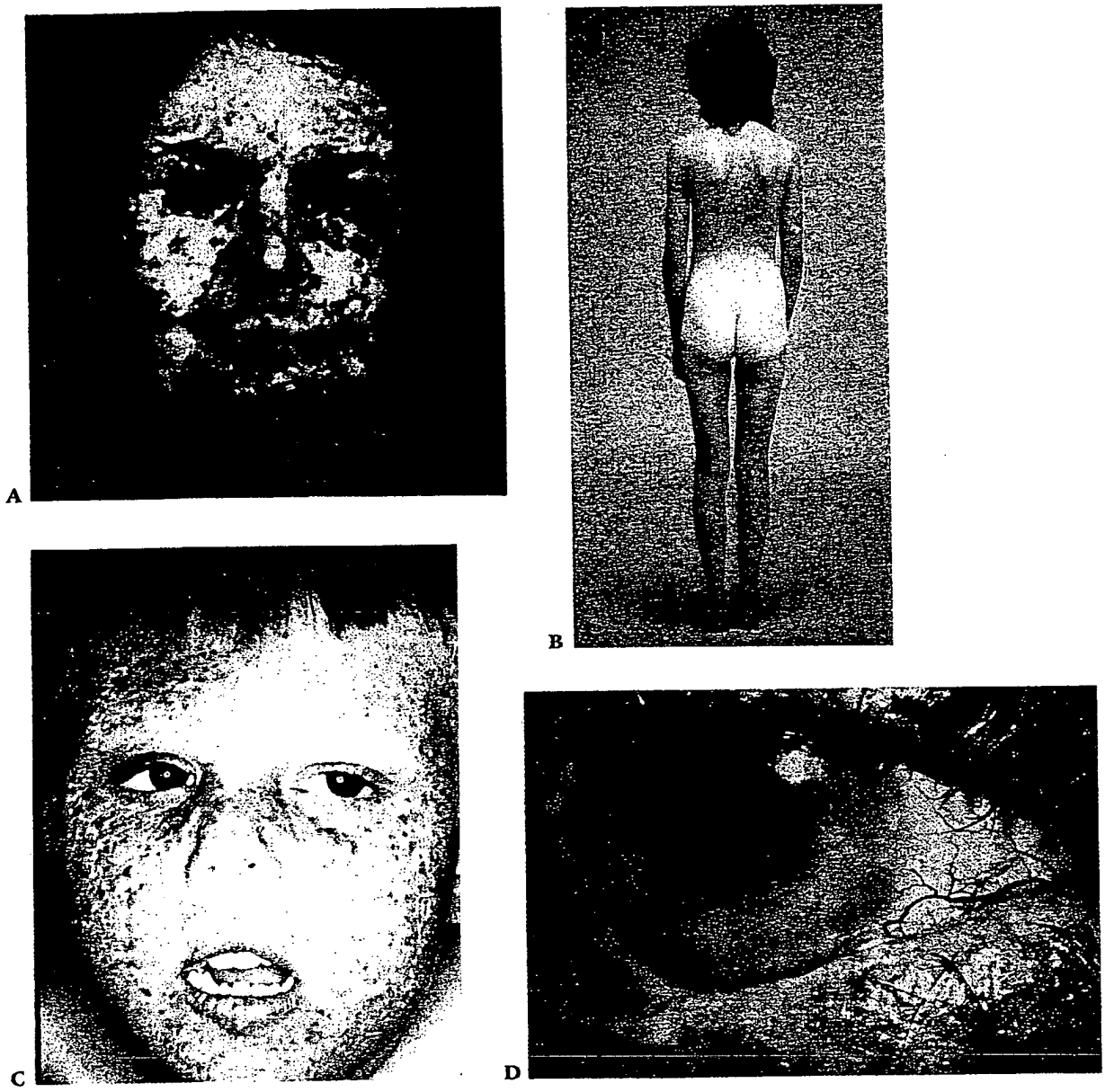
Color Plate 5 Energy-minimized structure for duplex DNA containing a thymine-thymine cyclobutane dimer. The structure has been oriented to display a helical kink of 27° and unwinding of 19.7° associated with the presence of the dimer (magenta). Sites of anticipated UvrABC-mediated endonucleolytic cleavage are shown at the eighth phosphodiester bond (red) 5′ to the dimer and the fourth or fifth phosphodiester bonds (red) 3′ to the dimer. (Reproduced with permission from B. Van Houten, H. Gamper, S. R. Holbrook, J. E. Hearst, and A. Sancar. 1986. Action mechanism of ABC excision nuclease on a DNA substrate containing a psoralen crosslink at a defined position. *Proc. Natl. Acad. Sci. USA* **83**:8077–8081. Photograph courtesy of A. Sancar.)

Color Plate 6 Space-filling model of DNA incorporating a psoralen cross-link, based on energy minimization structure. The cross-link unwinds the helix by 87.7° and produces a 46.5° helical kink. (Reproduced with permission from D. A. Pearlman, and S. R. Holbrook. 1985. Molecular models for DNA damaged by photoreaction. *Science* **227**:1304–1308. Photograph courtesy of A. Sancar.)

Color Plate 7 Mapping of the *XPG* gene to human chromosome 13q33 by fluorescence in situ hybridization. Both of the chromosomes showing the positive hybridization signal (red dots) are chromosome 13. (Reproduced with permission from S. Samec, T. A. Jones, J. Corlet, D. Scherly, R. D. Wood, and S. G. Clarkson. 1994. The human gene for xeroderma pigmentosum complementation group G (*XPG*) maps to 13q33 by fluorescence in situ hybridization. *Genomics* **21**:283–285. Photograph courtesy of R. Wood.)



Color Plate 8 Space-filling model of the *E. coli* DNA polymerase III β-subunit dimer with B-form DNA. One monomer is colored red, and the other is colored yellow. The radii of the spheres correspond to the van der Waals radii of the corresponding atoms. Hydrogen atoms are not explicitly displayed but manifest as increased radii for atoms to which they are bonded. The B-form DNA is shown passing through the hole in the β subunit dimer with no steric repulsions. (Reproduced with permission from X.-P. Kong, R. Onrust, M. O'Donnell and J. Kuriyan. 1992. Three-dimensional structure of the β subunit of *E. coli* DNA polymerase III holoenzyme: A sliding DNA clamp. *Cell* **69**:425–437. Photograph courtesy of John Kuriyan.)



Color Plate 9 Clinical features of xeroderma pigmentosum (XP). (A) The disease typically presents with severe pigmentary disturbances on the sun-exposed areas of the skin. (B) Note that areas not normally exposed to sunlight are not affected. (C) These clinical features can present at a very early age. (D) The eyes are also frequently affected. The individual shown here has clouding of the cornea and atrophy and loss of lashes from the lower eye lid. (Reproduced with permission from K. H. Kraemer. Progressive degenerative diseases associated with defective DNA repair: xeroderma pigmentosum and ataxia telangiectasia. p. 37–71. *In* W. W. Nichols and D. G. Murphy (ed.), *DNA repair processes, Symposia Specialists, Miami,* 1977).

Color Plate 10 (A and B) Frontal and side views of the face of a young boy with trichothiodystrophy (TTD). Note the sparse broken scalp hair, eyebrows and eyelashes, the thickened epicanthal folds, the protruding ears and the receding chin. (C) View of the trunk of this same patient. Note the large ichthyotic (fish-like) scales on the anterior part of the trunk and the atopic eczema in the armpits. (Reproduced with permission from V. H. Price, R. B. Odom, W. H. Ward, and F. T. Jones. 1980. Trichothiodystrophy: sulfur-deficient brittle hair as a marker for a neuroectodermal symptom complex. *Arch. Dermatol.* **166**:1375–1384. Photograph courtesy of V. Price.)

A

B

Color Plate 11 Some of the clinical features of ataxia telangiectasia (AT). (A) The conjunctiva of the eye of an individual showing telangiectasia (abnormal dilatation of blood vessels). (B) This 22-year-old patient has severe ataxia and is wheelchair bound. (Reproduced with permission from K. H. Kraemer. Progressive degenerative diseases associated with defective DNA repair: xeroderma pigmentosum and ataxia telangiectasia, p. 37–71. *In* W. W. Nichols and D. G. Murphy (ed.) *DNA repair processes. Symposia Specialists, Miami.* 1977.)

Color Plate 12 Patients with Bloom's syndrome (BS) typically have a butterfly distribution of light-induced capillary dilatation of the skin of the face. (Photograph courtesy of J. German.)

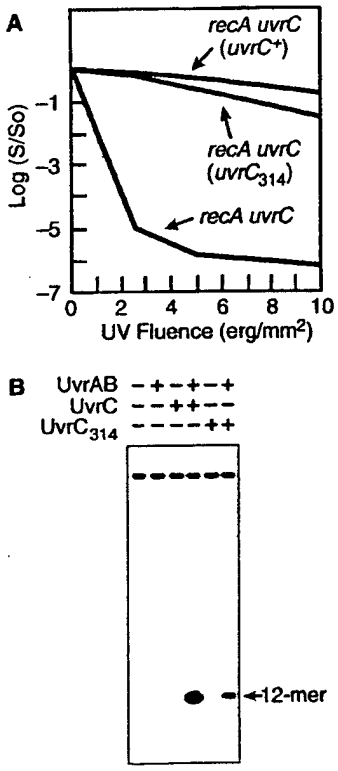


Figure 5–9 The C-terminal 314-amino-acid UvrC polypeptide is able to participate in nucleotide excision repair. (A) Transformation of a *recA uvrC* mutant with a plasmid that encodes the truncated UvrC protein restores resistance to UV radiation to levels comparable to that observed following transformation with a plasmid that encodes the wild-type UvrC protein (610 amino acids). (B) A 137-mer duplex DNA fragment containing ^{32}P radiolabel at the sixth phosphodiester bond 5′ to a psoralen monoadduct was incubated with the indicated proteins, and the reaction was analyzed on a DNA sequencing gel. Following autoradiography of the gel, an excised radiolabeled 12-mer oligonucleotide was observed as a product of the reactions with both the normal and the truncated UvrC protein. (*Adapted from Lin and Sancar [100].*)

cleases, in this case encoded by three distinct genes, have been implicated in nucleotide excision repair in the yeast *S. cerevisiae*.

These observations in *E. coli* provide a reasonable explanation for the observation of 3′-uncoupled incision of DNA with aged UvrC protein and with certain DNA substrates (102). Furthermore, the notion of uncoupled incisions provides a tenable explanation for the long-standing observation that *uvrC* mutant strains can still support some nicking of UV irradiated DNA, whereas *uvrA* and *uvrB* mutants cannot. The stoichiometry of incision by UvrB and UvrC proteins is one molecule of each protein per damaged site.

Incision is a relatively slow reaction which proceeds at a rate of $10^4\ M^{-1}s^{-1}$ (132). Like many of the biochemical events that precede incision, the incision step itself is dependent on the presence of ATP. However, in contrast to the formation of the $(UvrA)_2(UvrB)_1$ and $(UvrB)_1$-DNA complexes, the hydrolysis of ATP is not essential for DNA incision, and ATP[γS] can be substituted for ATP during this step (154).

Substrate Specificity of the UvrABC Endonuclease

In contrast to the largely nondistortive modifications of bases that are recognized by DNA glycosylases, the majority of the numerous chemicals to which *uvr* mutants are sensitive share with UV radiation the capacity for generating bulky base adducts which can cause significant distortion of the DNA helix (Table 5–3). One study has shown that a pyrimidine dimer unwinds the helix by 19.7° and introduces a kink of 27° which protrudes into the major groove of DNA (134). Additionally, one- and two-dimensional gel electrophoresis and quantitative electron microscopy studies of DNA fragments of known sequence containing thymine dimers in defined positions indicate that pyrimidine dimers cause a bend of ~30°

Table 5–3 Substrates for the UvrABC endonuclease of *E. coli*

DNA-damaging agent	Adduct(s)
N-acetoxy-2-acetylaminofluorene	*C*-8-Guanine
Anthramycin	*N*-2-Guanine
AP sites	Base loss
AP sites (reduced)	Ring opened AP site
Alkoxamine-modified AP sites	AP site analog
Benzo[*a*]pyrenediolepoxide	*N*-2-Guanine
CC-1065	*N*-3-Adenine
Cisplatin and transplatin	*N*-7-Guanine
Cyclohexylcarbodiimide	Unpaired G and T residues
Ditercalanium	Noncovalent bisintercalator
Doxorubicin and AD32	Intercalated compounds
N-hydroxyaminofluorene	*C*-8-Guanine
N,N'-bis(2-chloroethyl)-*N*-nitrosourea	Bifunctional alkylation
N-methyl-*N'*-nitro-*N*-nitrosoguanidine	O^6-Methylguanine
Mitomycin	*N*-7-Guanine
Nitrogen mustard	Bifunctional alkylation
4-Nitroquinoline-1-oxide	*C*-8, *N*-2-Guanine
	N-6-Adenine
6-4 Photoproduct	*C*-6, *C*-4-PyC
Psoralen	*C*-5, *C*-6-Thymine
Pyrimidine dimer	*C*-5, *C*-6-Pyrimidine
Thymine glycol	*C*-5, *C*-6-Thymine

Source: Adapted from Van Houten (210).

in DNA (75). There is also evidence for structural deformation of DNA containing the bulky monoadduct acetylaminofluorene (159).

DNA cross-links are particularly distortive. Space-filling models suggest that a psoralen cross-link unwinds the DNA duplex by 87.7°, causes a 46.5° helical kink, and displaces the helical axis by 3.49 Å (0.35 nm) (134) (Color Plate 6). This model has been largely confirmed by using two-dimensional nuclear magnetic resonance spectroscopy (NMR) (206) and electron microscopy (181). Finally, studies on DNA treated with a different cross-linking agent (cisplatin) indicate that the adduct *cis*-[$Pt(NH_3)_2$-(dpGpG)] kinks the DNA into the major groove by 50 to 60° and unwinds it by 40 to 60° (85).

There has been considerable emphasis on conformational distortion of DNA structure as a crucial element for damage-specific recognition leading to the productive incision of DNA. However, in vitro studies have demonstrated that the *E. coli* damage-specific endonuclease has a range of substrate specificity that includes base damage which is not considered distortive (138, 170, 210, 215). Thus, for example, lesions such as AP sites, thymine glycols, and O^6-methylguanine are recognized as substrates by the enzyme (84, 93, 138, 168, 170, 193, 199, 210, 215, 220). Single-nucleotide mismatches and even larger loops caused by mispairing, as well as naturally bent DNA, are not productive substrates for the UvrABC endonuclease, however (170). Intercalating agents such as ethidium bromide and chloroquine result in binding sites in DNA for UvrA protein, but the addition of UvrB and UvrC proteins does not lead to incision in these instances. In fact, trapping of UvrA protein in nonproductive protein-DNA complexes containing intercalated chemicals can result in the inhibition of nucleotide excision repair. This phenomenon possibly explains the well-known observation that caffeine is an inhibitor of DNA repair (169).

In general, these observations are consistent with the suggestion that many types of base damage are recognized with differing affinities by the UvrABC endonuclease and that the K_m for binding varies as a function of the type and extent of helix distortion produced. Alternatively, as discussed above, perhaps productive binding of UvrB protein to DNA results only when base damage precludes the adoption of specific conformational constraints imposed by the protein on native

DNA, thereby freezing the protein-damaged DNA complex in place, or when UvrB protein specifically recognizes alterations in base-stacking interactions in DNA. Earlier in the chapter, we referred to the presence of a glycine-rich region at the C terminus of the UvrA protein. Glycine and alanine residues allow for more accessible conformations of proteins (106). Hence, it has been suggested that this feature of UvrA protein might facilitate a flexible induced-fit mechanism for the specific recognition of many different types of base damage (62).

Roles of ATP in Damage-Specific Incision

It is evident from the preceding discussion that ATP is essential for damage-specific incision during nucleotide excision repair in *E. coli* (62, 64, 152, 210). This high-energy compound appears to play several roles in this process, reflecting the fact that ATP is not only a source of energy but is also a ligand whose binding causes conformational changes in the Uvr proteins. The key steps in which ATP participates can be summarized as follows.

(i) ATP increases the dimerization constant of the UvrA subunit and is apparently bound rather than hydrolyzed in this process, since nonhydrolyzable ATP analogs can substitute for ATP.

(ii) ATP is essential for the formation of the $(UvrA)_2(UvrB)_1$ complex. Nonhydrolyzable ATP analogs cannot substitute for ATP in this event. Hence, more than simple binding of ATP is apparently involved here.

(iii) ATP is necessary for the formation of a specific $(UvrB)_1$-DNA complex.

(iv) Some form of a UvrAUvrB complex [possibly $(UvrA)_2(UvrB)_1$] hydrolyzes ATP in order to drive a DNA helicase activity.

(v) ATP is necessary for the bimodal incision of DNA.

Role of Proteolysis in the Regulation of DNA Incision by the UvrABC Endonuclease

As already indicated, a proteolysis product of UvrB protein has been detected in cell extracts of *E. coli*. This degradation product, UvrB* protein (24),is capable of interacting with UvrA protein and exhibits a DNA-dependent ATPase activity. However, the UvrAUvrB* complex is devoid of DNA helicase-ATPase activity and does not facilitate incision of DNA in the presence of UvrC protein (24). A protease that cleaves UvrB protein in vitro has been identified as OmpT protease, which is also involved in the cleavage of Ada protein (see chapter 3) (6, 161). It has been suggested that specific proteolysis of UvrB protein might regulate incision of DNA in vivo, thereby protecting the genome from spurious cutting of undamaged DNA if the intracellular levels of Uvr proteins are abnormally elevated for any reason. Additionally, or alternatively, proteolysis of UvrB protein in vivo might conceivably facilitate turnover of the UvrB-UvrC-DNA complex following damage-specific incision and of unproductive complexes that are improperly constituted or bind to sites that cannot be cleaved (24). However, many proteins have been observed to be cleaved by the OmpT protease in vitro during cell lysis (70), and at present there is no convincing biological evidence in support of proteolysis as a mechanism for regulating nucleotide excision repair in *E. coli*.

Summary of Damage-Specific Incision during Nucleotide Excision Repair in *E. coli*

Damage-specific incision of DNA is a biochemically complex process, even in a simple prokaryote like *E. coli*. This complexity derives from the stochastic nature of base damage to DNA. As a consequence, living cells cannot rely on DNA sequence for the specificity of protein-DNA interactions and have evolved an elaborate multistep process during which the ordered assembly of DNA-protein complexes provides an architectural specificity which discriminates damaged from undamaged sites. Much is still to be learned about precisely how this specificity is achieved, and the UvrABC endonuclease of *E. coli* will certainly continue to serve as a primary instructional model for understanding the detailed biochemical mechanisms of nucleotide excision repair.

Postincisional Events—Excision of Damaged Nucleotides, Repair Synthesis, and DNA Ligation

The demonstration of a bimodal incision mechanism led to the realization that the actual excision of base damage during nucleotide excision repair in *E. coli* requires the release of the oligonucleotide fragment defined by these incisions and of the bound UvrB and UvrC proteins. The UvrBUvrC complex does not turn over at a detectable rate in vitro (25, 152, 170). However, such turnover clearly takes place in living cells. For example, when UV-irradiated *E. coli* cells are held in liquid after exposure to UV radiation (*liquid holding recovery*), thousands of pyrimidine dimers are excised over several hours (200).

The phenomenon of liquid holding recovery is not observed in *E. coli* mutants which are defective in the *polA*$^+$ or *uvrD*$^+$ genes (200). This observation prompted an examination of Pol I and UvrD proteins in the presence of the UvrABC proteins. Purified Pol I and UvrD proteins increase the extent of DNA incision, consistent with increased turnover of the UvrBUvrC incision complex (25, 77, 89). It is not clear whether both proteins are absolutely required for this effect, since stimulation can be achieved with just one or the other under certain experimental conditions (170). However, maximal stimulation requires both Pol I and UvrD proteins.

These observations are supported by biological studies. Whereas *uvrD* mutants of *E. coli* are not totally defective in DNA incision, these mutants display abnormally low rates of incision in semi-in vivo experimental systems, e.g., in cells permeabilized with detergents (11). In addition, intact UV-irradiated *uvrD* mutant cells manifest a reduced rate of excision of pyrimidine dimers (87, 140, 141, 216) (Fig. 5–10). Consistent with an auxiliary role of UvrD protein in nucleotide excision repair, mutant strains that are entirely defective in UvrD protein are not as sensitive to UV radiation as are *uvrA*, *uvrB*, or *uvrC* mutants (225) (Fig. 5–10).

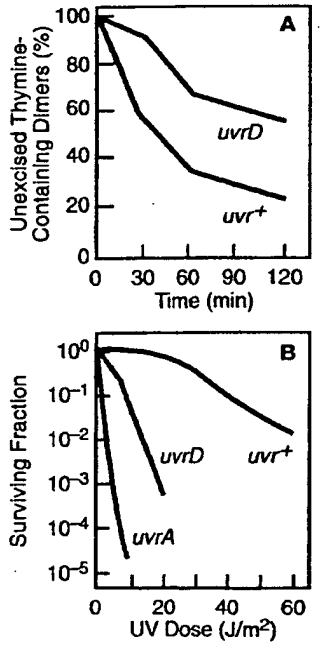


Figure 5–10 (A) Mutants defective in the *uvrD* gene show a reduced rate and extent of loss of thymine-containing pyrimidine dimers from DNA. (B) Such mutants are also abnormally sensitive to UV radiation, but they are not as sensitive as mutants defective in the *uvrA* gene. (*Adapted from Friedberg [45a] with permission.*)

Role of UvrD Protein (DNA Helicase II) and Pol I in Excision and Repair Synthesis

THE *uvrD*$^+$ GENE

The gene that encodes UvrD protein was independently identified in a number of different studies and hence was initially given redundant genetic designations. A UV-sensitive mutant of *E. coli* was shown to be distinct from mutants defective at the *uvrA*, *uvrB*, and *uvrC* loci, and the relevant gene was designated *uvrD*$^+$ (125). Subsequently a strain containing a mutant allele called *mutU* which conferred increased UV radiation sensitivity and an increased spontaneous mutation frequency, was isolated (186). At about the same time, other mutant alleles called *uvrE*, which conferred very similar phenotypes, were identified (190), and yet another locus that affects genetic recombination was designated *recL* (72). This genetic complexity was resolved when it was shown that *uvrE*, *recL*, *mutU*, and *uvrD* are all alleles of the same gene, which is located at about 84 min on the *E. coli* genome (92).

The *uvrD*$^+$ gene has been cloned (7, 103, 123), and plasmid or phage vectors carrying the gene complement the UV radiation sensitivity of recessive *uvrD*, *uvrE*, and *recL* mutations (7, 103, 123). Analysis of the polypeptides expressed from these vectors has identified the product of the *uvrD*$^+$ gene as a single polypeptide of ~75-kDa, with a DNA-dependent ATPase activity (7, 88, 91, 103, 123, 124). A known DNA-dependent ATPase of *E. coli* with the same molecular mass was previously designated as DNA helicase II (91). It is now firmly established that UvrD protein and DNA helicase II are one and the same, and henceforth we shall refer to this protein as *DNA helicase II*.

Like the *uvrA*$^+$ and *uvrB*$^+$ genes, the *uvrD*$^+$ gene is inducible by DNA damage. When *E. coli* cells are treated with mitomycin or nalidixic acid (well-established inducers of SOS-regulated genes [see chapter 10]), the level of DNA-dependent ATPase activity increases four- to sixfold (88). This increase is not observed in *recA* mutants defective in the SOS response (103). Additionally, the promoter region

of the *uvrD*$^+$ gene has been shown to contain a canonical LexA protein-binding site (91).

The addition of Pol I and DNA helicase II to the UvrABC endonuclease-mediated reaction in vitro does not alter the initial rate of DNA incision. This rate (~0.3 incision per min) is apparently determined by the kinetics of assembly of the UvrBUvrC complex, after which a lower secondary rate (~0.1 incision per min) is established. This lower rate is the product of multiple reactions, some of which involve Pol I and DNA helicase II proteins and include displacement of UvrB and UvrC proteins, dissociation of the oligonucleotide, repair synthesis of DNA, and the reloading of Uvr proteins on the DNA at new sites of damage (Fig. 5–11). This second steady-state rate results in the excision of ~0.08 dimers per min, a rate that reasonably approximates the rate measured in vivo (~0.25 dimer excised per min) (77, 152).

The precise mechanism by which Pol I and DNA helicase II effect the turnover of UvrB and UvrC proteins and displacement of the damage-containing oligonucleotide is not clear. Footprinting studies with the Uvr proteins plus Pol I and DNA helicase II do not reveal the presence of a large multiprotein complex. Therefore, there is no experimental evidence in support of a stable large protein machine ("repairosome") comprising the UvrB and UvrC proteins, Pol I, DNA helicase II, and possibly DNA ligase (152). However, DNA footprinting would not detect short-lived complexes, and such a large multiprotein complex may exist transiently.

DNA helicase II is required for the release of the oligonucleotide fragment and of UvrC protein from the postincision complex. Other *E. coli* DNA helicases

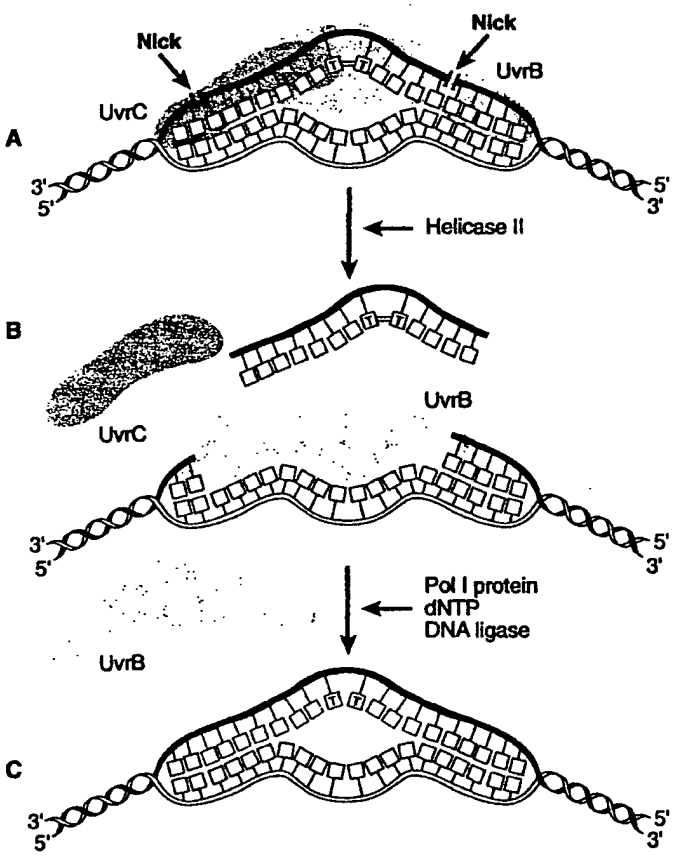


Figure 5–11 Model for postincisional events during nucleotide excision repair in *E. coli*. DNA helicase II (UvrD protein) is required for the release of an oligonucleotide fragment (excision) following bimodal incisions generated by the UvrABC endonuclease and for the displacement of UvrC protein (A and B). UvrB protein remains bound to the gapped DNA during the excision reaction and is released during the repair synthesis reaction catalyzed by Pol I (C). DNA ligation completes the nucleotide excision repair reaction (C).

cannot substitute for this function, suggesting that this is a specific function of DNA helicase II (132). It is not known whether these displacement reactions require that DNA helicase II specifically interact with the Uvr proteins in the postincision complex or with the nicks that are generated during the incision process. The latter possibility is certainly feasible, since studies with purified DNA helicase II have demonstrated its ability to initiate the unwinding of duplex regions at nicks when it is present in sufficiently high concentrations (143, 144). UvrB protein is not displaced during the excision reaction but apparently remains bound to the gapped DNA (132) and is released only when Pol I and substrate deoxynucleoside triphosphates (dNTPs) for repair synthesis are present (Fig. 5–11). It appears, then, that following incision, DNA helicase II releases UvrC protein and the damage-bearing oligonucleotide, leaving a UvrB–gapped-DNA complex in which UvrB protein presumably protects the single-stranded DNA from nonspecific degradation. Pol I binds to the 3′ OH terminus generated at the 5′ incision and displaces bound UvrB protein during the course of repair synthesis (132) (Fig. 5–11).

When Pol I and DNA helicase II are present in an excision repair reaction together with DNA ligase, a repair patch of 12 nucleotides is observed ~90% of the time. The repair patch size has been accurately determined in vitro by a novel

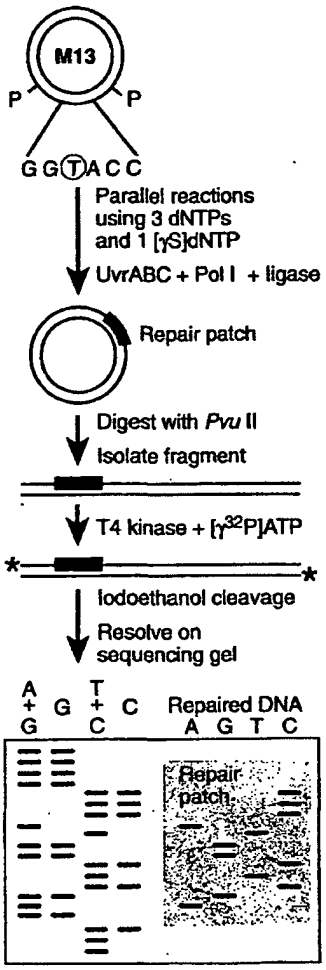


Figure 5–12 *Assay to measure the size of the repair patch generated during in vitro nucleotide excision repair in E. coli.* The plasmid DNA substrate contains a single psoralen adduct (circled T) in a defined position contained within a *Pvu*II (P) restriction fragment. This substrate is incubated in four separate reactions, each containing the UvrABC endonuclease, *E. coli* Pol I, DNA ligase, three unlabeled dNTPs, and one dNTP[γS]. The dNTPs and dNTP[γS] are varied between reactions. Following the reaction the DNA is digested with *Pvu*II. The resulting 322-bp DNA fragment is end labeled with polynucleotide kinase and heated in the presence of iodoethanol. This compound ethylates the phosphorothioate groups incorporated during repair synthesis and makes them sensitive to hydrolysis at high temperature. The DNA fragment is then analyzed on a sequencing gel, generating a sequence ladder of the repair patch. The sequence of the entire repair patch can be unambiguously read, and its size can be accurately measured. (*Adapted from Sibghat-Ullah, et al. [185]*).

and elegant technique for measuring repair synthesis (185) (Fig. 5–12). A plasmid substrate containing a single psoralen monoadduct in a unique location was used as the substrate for nucleotide excision repair in the presence of UvrABC endonuclease, Pol I, and DNA ligase (without added DNA helicase II). Repair synthesis is carried out in the presence of a dNTP carrying sulfur instead of phosphorus in the α position, resulting in a DNA product with phosphorothioate bonds. These bonds are preferentially cleaved by heating the DNA in the presence of iodoethanol. Hence, if a restriction fragment of the DNA bearing the entire repair patch is end labeled with ^{32}P, sites of phosphorothioate cleavage can be identified on a DNA sequencing gel. When repair synthesis is carried out in four sequential reactions, each in the presence of a single thiotriphosphate, the composite sequence ladder will be confined to the region of repair synthesis.

From these results it has been concluded that nick translation catalyzed by the 5′ → 3′ exonuclease of *E. coli* Pol I is not required during nucleotide excision repairs in vitro. Several factors have been implicated in the generally strict avoidance of nick translation during repair synthesis. For one thing, it turns out that the excision gap size of ~12 nucleotides is about the length of the optimal processivity of Pol I (222). It has also been suggested that DNA helicase II may move with Pol I in the direction of repair synthesis (but on the opposite strand), and may somehow facilitate displacement of Pol I once the gap is filled (154).

The failure to observe nick translation during repair synthesis associated with nucleotide excision repair is at first glance difficult to reconcile with the observation that mutants (called *polAex*) which are defective in the 5′ → 3′ exonuclease function of Pol I are abnormally sensitive to UV radiation (34). However, it would appear that this phenotype is not the result of defective repair synthesis associated with defective nick translation but, rather, stems from qualitatively abnormal repair synthesis by the Pol I encoded by the *polAex* allele, resulting in a frequent failure to insert the last nucleotide during the gap-filling process and, at a lower frequency, in strand displacement which allows for prolonged synthesis by the *polAex* polymerase (222) (see the next section). Another possible explanation for the UV radiation sensitive phenotype in *polAex* mutants is that the 5′ → 3′ exonuclease function of *E. coli* Pol I plays a role in some other aspect of excision repair. One such possible role is suggested by the phenomenon of long patch excision repair.

LONG-PATCH EXCISION REPAIR OF DNA

A small fraction of the repair synthesis patches in *E. coli* are much longer than 12 nucleotides and can in fact be longer than 1,500 nucleotides (35, 86). The process whereby these tracts are generated is referred to as *long patch excision repair* to distinguish it from the more general *short patch repair mode* (36) associated with the conventional nucleotide excision repair pathway which is the central theme of this chapter. The observation of long repair patches implies that under some circumstances repair synthesis is preceded or accompanied by extensive degradation of DNA.

Long-patch repair differs from short patch repair in its absolute requirement for the *SOS inducible response* (35) already alluded to on several occasions and presented fully in chapter 10. Independent evidence for an inducible pathway of nucleotide excision repair comes from the observation that the completion of at least some fraction of the repair events initiated by the *uvrA*$^+$, *uvrB*$^+$, and *uvrC*$^+$ genes in vivo requires the *recA*$^+$ and *lexA*$^+$ genes, as well as the capacity for protein synthesis (233).

The frequency of long patches during nucleotide excision repair is increased in certain *uvrD* mutants (140) and in mutants defective in the 5′ → 3′ exonuclease function of Pol I (34). The molecular mechanism and physiological significance of normally occurring long patch excision repair are not known. In *E. coli* cells in which the SOS system is induced, this excision repair mode correlates with a more rapid recovery of semiconservative DNA synthesis and with cell survival after UV irradiation, compared with uninduced cells (68). Thus, this mode of repair syn-

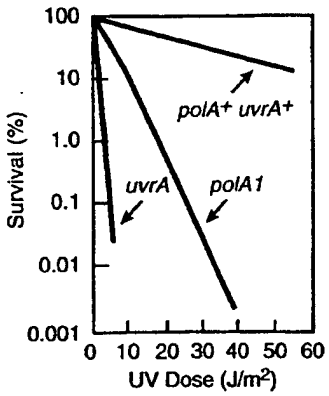


Figure 5–13 *E. coli polA* mutants are abnormally sensitive to killing by UV radiation. However, they are not as sensitive as *uvrA* mutants. (*Adapted from Friedberg [45a] with permission.*)

thesis may reflect a distinct form of nucleotide excision repair associated with the ability of *E. coli* to bypass pyrimidine dimers at or near replication forks (see chapter 11).

It is of historical interest that long-patch repair was discovered by the paradoxical observation of increased total repair synthesis of DNA in UV-irradiated cells which are defective in Pol I, despite the repair of fewer pyrimidine dimers in these mutants (37). This led to the speculation that the primary biochemical distinction between short- and long-patch repair is that the former is mediated by Pol I and the latter is mediated by other DNA polymerases (37). However, strains defective in Pol II or III carry out essentially normal amounts of long-patch repair under conditions where this repair synthesis mode is optimally observed (see below) (35). It appears, then, that Pol I is required for both short- and long-patch repair.

Repair Synthesis

A description of the *E. coli* DNA polymerases was presented in the previous chapter. The complete absence of repair synthesis of DNA in UV-irradiated *E. coli* is observed only in mutants defective in all three DNA polymerases, suggesting that all of these enzymes are potentially able to perform this function (104). This is consistent with the observation that in vitro all three enzymes can utilize gapped DNA substrates of the type generated during nucleotide excision repair. It is therefore difficult to assess the relative contribution of each to repair synthesis in wild-type cells. Nonetheless, several observations suggest that *E. coli* Pol I occupies a primary role in repair synthesis under normal conditions. First, *polA* mutants are abnormally sensitive to UV radiation (57), although, not surprisingly in view of the redundancy just described, these mutants are not as sensitive as *uvrA*, *uvrB*, or *uvrC* mutants (Fig. 5–13). Second, *polB* mutants, which are defective in Pol II, are not abnormally UV sensitive (22), and Pol II cannot substitute for Pol I in the turnover of UvrB protein. Since *polC* mutants (defective in DNA Pol III) exist only as conditional-lethal mutants, it is not possible to assess their UV sensitivity under conditions in which the enzyme is not functional (69). However, the participation of Pol III in dimer excision in *E. coli* is suggested by the observation that a mutant (*polA polC*) deficient in both Pol I and III has much less excision capability than does the *polA* strain alone, even at low UV doses (38).

DNA Ligation

The final postincisional biochemical event in all forms of excision repair is the joining of the last newly incorporated nucleotide to the polynucleotide chain, i.e., the sealing of the nick left following the completion of repair synthesis. In *E. coli*, this event is catalyzed by an extensively characterized enzyme called DNA ligase (see chapter 4).

Nucleotide Excision Repair of Cross-Links in DNA—a Special Situation

The excision of cross-links in DNA presents a special problem, since adducts are covalently attached to both DNA strands. Early genetic studies revealed a requirement for the *recA*$^+$ gene of *E. coli*. In addition to its regulatory role in the SOS response, the product of this gene, RecA protein, is involved in recombination (see chapters 10 and 11). This suggested that the dilemma of avoiding cell death while having to cut both strands of the genome might be solved by recombinational events (187, 188, 210). An incisional-recombinational model proposed that the UvrABC complex effects the incision of one DNA strand, generating an oligonucleotide that remains covalently attached to DNA through the cross-link (Fig. 5–14). Displacement of the oligonucleotide would then generate a gapped structure which could be repaired by recombinational repair (see chapter 11). Subsequent incision on the other strand would result in release of a cross-linked oligonucleo-

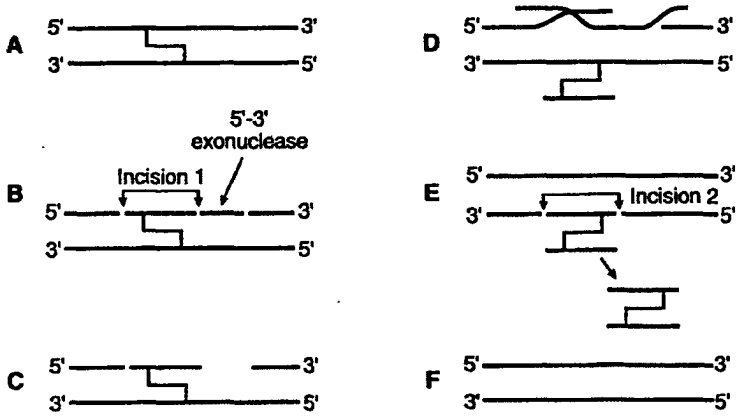


Figure 5–14 Model for the repair of DNA interstrand cross-links. (A) Cross-linked DNA. (B) The UvrABC endonuclease cuts just the furan strand of the DNA, generating incisons which flank the cross-link (incision 1). (C) It is proposed that the 5′ → 3′ exonuclease of Pol I then generates a gap 3′ to the site of the cross-link, which is a substrate for RecA-mediated recombination. (D) During recombinational repair of the gap, an invading homologous DNA strand displaces the cross-linked oligonucleotide. (E) Once recombination is completed, the pyrone DNA strand is incised by the UvrABC endonuclease (incision 2), leading to the excision of a complex 11-mer or 12-mer oligonucleotide structure. (F) DNA repair is completed by repair synthesis and ligation. (*Adapted from Van Houten [210].*)

tide structure, leaving a second gap to be repaired by conventional repair synthesis and DNA ligation (33) (Fig. 5–14).

Biochemical experiments have provided considerable support for this model. As indicated previously, studies with purified Uvr proteins have shown that incision of psoralen cross-links does indeed occur exclusively on the furan strand (212), although other studies have shown that the incised strand is sometimes the pyrone strand (79). The UvrABC endonuclease is also able to incise a model three-stranded structure in which one of the strands is an oligonucleotide covalently attached to double-stranded DNA (28, 29). Additionally, in the presence of purified RecA protein and a "transiently" positioned third strand at the cross-linked site (provided by an oligonucleotide homologous to the furan side strand), the UvrABC endonuclease catalyzes incision of the pyrone side strand (27). Further support for the model described above derives from in vitro studies (189) showing that RecA protein can mediate repair of a cross-link if there is a gapped structure adjacent to the cross-linked DNA. The cross-link is first incised bimodally by the UvrABC endonuclease on one strand, and the gap flanking the incised region is generated in vitro by exonucleolytic degradation with the 5′ → 3′ exonuclease of Pol I. The gap is required for the initiation of RecA polymerization, and the final RecA recombinant product includes the short triple-stranded region due to the presence of the cross-link (189).

The incisions that accompany excision repair of interstrand cross-links have been analyzed with DNA containing psoralen. Thymine-psoralen-thymine cross-links are asymmetrical because one strand is linked via the furan ring of the psoralen while the other strand is attached to the pyrone ring (152) (Fig. 5–15). Incision of this lesion occurs at the ninth phosphodiester bond 5′ to the furan adducted strand and at the third bond 3′ to the adduct on the same strand, while the pyrone strand remains intact (212) (Fig. 5–15). With psoralen monoadducts there is no apparent preference for the furan ring. Hence, the unique incision reaction observed with psoralen cross-links apparently reflects an affinity imposed by the conformation of the cross-link (152). The mechanism of incision of DNA cross-linked with psoralen may be influenced by sequence contexts. Enrichment of the G+C content of the region immediately 5′ to the modified thymine residue

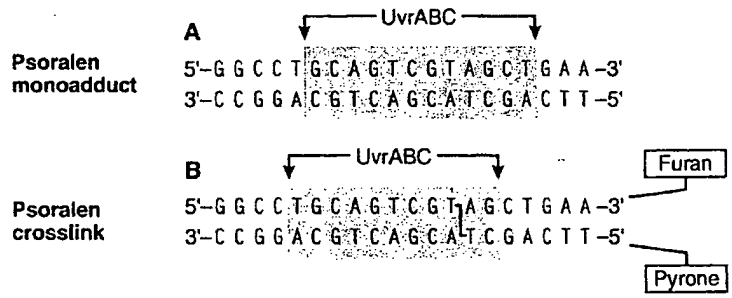


Figure 5–15 The incisions that accompany nucleotide excision repair of psoralen monoadducts and interstrand cross-links. (A) Incision of monoadduct base damage by the UvrABC endonuclease occurs at the eighth phosphodiester bond 5′ and the fifth phosphodiester bond 3′ to the lesion, on the strand containing the base damage. (B) A psoralen cross-link is cut at the ninth phosphodiester bond 5′ and the third phosphopdiester bond 3′ to the cross-link only on the furan-adducted strand, while the pyrone-adducted strand remains intact. (*Adapted from Van Houten, et al. [212].*)

on the furan strand results in preferential incision of that strand. However, when the 3′ side of this lesion is enriched for G + C, incision occurs on either strand (80).

The incision of cross-linked DNA by the UvrABC endonuclease is also influenced by the superhelical density of substrate DNA. The rate of incision of supercoiled DNA containing the furan monoadduct of psoralen is relatively insensitive to superhelix density. However, efficient incision of cross-linked (by psoralen) DNA requires underwound DNA (114). The $(UvrA)_2(UvrB)_1$ complex binds with equal efficiency at the site of both monoadducts and cross-links. Hence, the requirement for negative supercoiling for efficient nicking at the latter site must be at a step subsequent to lesion recognition (114). It has been pointed out that the formation of an underwound structure could not occur if either of the DNA strands was already nicked in the immediate vicinity. Hence, such underwinding provides a potential mechanism for avoiding the formation of double-strand breaks (114).

A Uvr Protein-Independent Pathway for the Repair of Cross-Links in DNA?

Repair of psoralen cross-links in DNA has been demonstrated to occur in *uvr* mutants of *E. coli* (19–21). Additionally, an *E. coli* mutant with enhanced resistance to photoactivated psoralen has been isolated and shown to overproduce a 55-kDa protein (2), and a novel enzymatic activity which exclusively incises DNA containing psoralen monoadducts has been identified in *E. coli* (189, 210). Preliminary characterization of this activity suggests that it might be a DNA glycosylase (210).

Coupling of Transcription and Nucleotide Excision Repair in *E. coli*

Studies first carried out with mammalian cells demonstrated that nucleotide excision repair of DNA occurs preferentially in actively transcribed genes (see chapters 7 and 8). A similar phenomenon has been demonstrated in *E. coli*, for which it has been shown that in actively transcribed DNA nucleotide excision repair has a pronounced bias for the transcribed strand (108). These observations led to the initial suggestion that transcriptional arrest caused by the presence of bulky adducts in DNA may serve as a signal for the binding of Uvr proteins, leading to the preferential repair of the template strand in genes which are presumably important for cell survival, since they are transcriptionally active (108). This could not only explain the bias for the transcribed strand but also provide an additional structural element for evaluating the complex issue of substrate recognition discussed above.

The phenomenon of transcriptional arrest by base damage in template strands of DNA has been extensively described (109, 157). When the "stalled RNA

polymerase" hypothesis was directly tested by adding purified *E. coli* RNA polymerase to nucleotide excision repair reactions in the presence of UvrA, UvrB, and UvrC proteins, it was paradoxically observed that the presence of the polymerase actually inhibited nucleotide excision repair (171). A search for an activity that could both overcome this inhibition and direct strand-specific repair in a cell-free system led to the discovery of a protein designated *transcription-repair coupling factor* (TRCF) in extracts of *E. coli* (172).

TRCF is the product of a gene called *mfd*$^+$ (for mutation frequency decline), which maps at 25.3 min on the *E. coli* chromosome (173,174). For improved understanding of mutation frequency decline and how it relates to strand-specific repair, we must briefly recount some key observations which date back to the mid-1950s. Almost exactly 35 years prior to the discovery of TRCF, Witkin (227) reported the curious observation that wild-type strains of *E. coli* manifest a decrease in the frequency of DNA damage-induced mutations when protein synthesis is transiently inhibited immediately after exposure of the cells to DNA damage. She subsequently noted that this phenomenon is dependent on the *uvr*$^+$ genes and on the *mfd*$^+$ gene (defined by the isolation of an *mfd* mutant strain) (228). The *mfd* mutant was found to have a distinctly lower rate of excision of pyrimidine dimers from DNA (53), suggesting that mutation frequency decline might involve a specialized form of nucleotide excision repair.

The phenomenon of mutation frequency decline and the function of the *mfd* gene were largely ignored, except for a series of provocative experiments reported by Bockrath and his colleagues during the 1970s, the details of which are well reviewed elsewhere (174). These experiments led Bockrath and his collaborators to conclude that "MFD is a unique process involving excision repair of premutational lesions located only in the transcribed strand of DNA" (15). Remarkably, this conclusion was proffered 12 years before strand-specific repair of DNA was formally described in *E. coli*.

Direct examination of extracts of *mfd* mutant cells led to the observation that they are defective in strand-specific repair and that this defect can be corrected by the addition of partially purified TRCF (175) (Table 5–4). Further support for the conclusion that the *mfd*$^+$ gene encodes TRCF came from several studies demonstrating that whereas UV radiation-induced mutations at sites of adjacent pyrimidines in the *lacI*$^+$ gene arise largely in the nontranscribed strand in *mfd*$^+$ strains, they arise largely in the transcribed strand in *mfd* mutant cells (90, 129).

The *mfd*$^+$ gene was enriched from an *E. coli* genomic library by PCR with degenerate oligonucleotide primers deduced from the N-terminal amino acid sequence of partially purified TRCF protein, and was cloned by functional complementation of a UV-sensitive *mfd* mutant (173) (Fig. 5–16). The cloned gene can encode a protein of ~130 kDa. The translated amino acid sequence of the *mfd*$^+$ gene reveals the presence of multiple consensus domains observed in many nu-

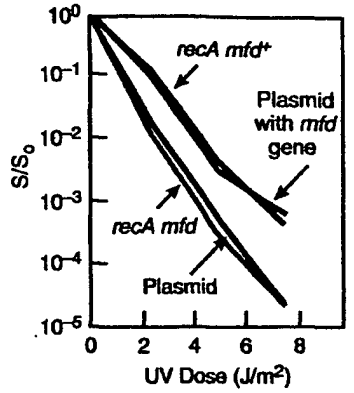


Figure 5–16 Phenotypic complementation of the UV radiation sensitivity of an *E. coli mfd* mutant by introduction of the cloned *mfd* gene. (*Adapted from Selby and Sancar [173] with permission.*)

Table 5–4 Repair synthesis in the transcribed and coding strands of DNA

Strain	Strand[a]	Repair synthesis[b]		
		With Rif	Without Rif	Without Rif, with TRCF
WU3620 (*mfd*$^+$)	t	111	470	575
	c	100	103	147
	t/c ratio	1.1	4.5	3.9
WU3610-45 (*mfd-1*)	t	111	116	351
	c	100	117	145
	t/c ratio	1.1	1.0	2.4

[a] t, transcribed; c, coding.

[b] Repair synthesis is shown in *mfd*$^+$ and *mfd* mutant strains of *E. coli* in the presence or absence of rifampicin (Rif) and TRCF. The t/c ratio reflects the strand preference for repair.

Source: Adapted from Selby, et al. (175).

cleotide binding proteins. Purified TRCF is indeed a weak ATPase (k_{cat} = ~3 min^{-1}), but the ATPase activity is not DNA dependent and the purified protein does not have detectable DNA helicase activity in isolation (173). Mfd protein also has a 140-amino-acid stretch near the N terminus which is homologous with *E. coli* UvrB protein and related proteins from several other prokaryotes, and it also has a leucine zipper motif near the C terminus (173). It has been estimated that there are about 500 copies of TRCF protein per cell (174). The protein is a monomer and binds weakly to DNA.

Experiments with purified TRCF, Uvr proteins, and RNA polymerase suggest that TRCF is able to recognize and interact with a stalled RNA polymerase–damaged DNA–mRNA ternary complex, resulting in displacement of the stalled polymerase and the truncated transcript and in binding of TRCF to DNA at or near the site of base damage. TRCF has a demonstrated binding affinity for UvrA protein, suggesting that when bound to damaged DNA it might be especially efficient in recruiting $(UvrA)_2(UvrB)_1$ protein complexes to DNA and in facilitating the formation of productive UvrB-DNA complexes. This results in strand-selective nucleotide excision repair (Fig. 5–17) (173).

In summary, in *E. coli* (and as we shall see in later chapters, in higher organisms) there are at least two modes of nucleotide excision repair. One mode does not involve the coupling of repair to transcription and is used for nucleotide excision repair of transcriptionally silent genes and presumably for the repair of the nontranscribed strand of transcriptionally active genes. A second mode appears to be mechanistically very similar, if not identical, but has a specific requirement for a protein which can target the nucleotide excision repair machinery to sites of base damage in the transcribed strand with a high degree of preference. Mutants defective in the *mfd* gene have a significant but limited sensitivity to UV radiation damage (129, 173) which is less than that exhibited by *uvr* mutants. This may explain the fact that mutants selected on the basis of reduced survival after exposure to DNA damage were readily related to nucleotide excision repair, independent of a strand bias for such repair. Had nucleotide excision repair-defective mutants been originally selected on the basis of a phenotype of DNA damage-dependent mutation frequency, the history of our understanding of this DNA repair mode might be quite different.

Miscellaneous Functions Possibly Associated with Nucleotide Excision Repair

The involvement of other functions in nucleotide excision repair (either prior to, concomitant with, or following DNA incision) is suggested by a number of interesting observations which have not yet been fully explored. For example, mutants of *E. coli* with mutations in a gene designated *top*$^+$, which encodes DNA topoisomerase I, are abnormally sensitive to killing by UV radiation (194). In addition, the presence of pyrimidine dimers in DNA is associated with a reduction in the rate of the relaxation of supercoiled plasmid DNA by *E. coli* DNA topoisomerase I in vitro (135). This has led to the suggestion that altered relaxation of damaged superhelical DNA in vivo resulting from reduced topoisomerase I activity might inhibit recognition and binding of the UvrABC endonuclease to damaged DNA if this enzyme is sensitive to the superhelical density of substrate DNA (135). Other DNA topoisomerases such as DNA gyrase might also participate in nucleotide excision repair of DNA in some kinetically relevant fashion. Little is known about the role of DNA topology in DNA repair, and it is hoped that future years will provide enlightening information in this area, although it is unlikely that DNA topology plays a crucial mechanistic role.

Are Uvr Proteins Involved in Other Cellular Processes in *E. coli*?

As indicated above, *uvrB* mutants are inviable in the presence of mutations in the *polA* gene (111). Similar observations have been made with *uvrD polA* (111) and *uvrA polA* double mutants (94). These observations await a definitive explanation.

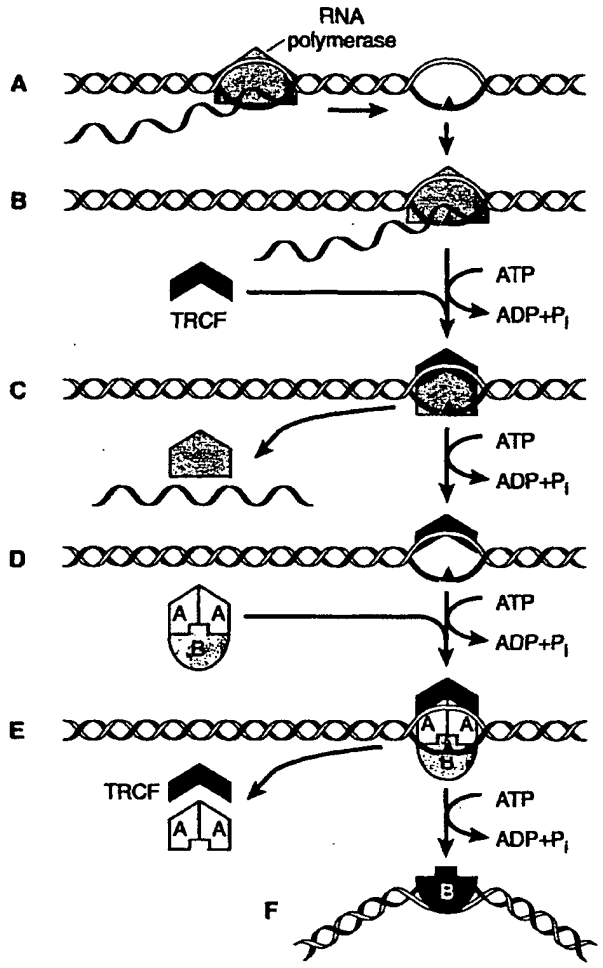


Figure 5–17 Model for strand-specific nucleotide excision repair in *E. coli*. (A) RNA polymerase is shown transcribing a template DNA strand which contains base damage ahead of the transcription complex. (B to D) Stalling of RNA polymerase at the site of base damage in the transcribed strand (B) results in the binding of TRCF (C) and displacement of the polymerase and the truncated transcript, leaving TRCF bound at the site of damage (D). (E and F) TRCF binds to UvrA protein, resulting in the recruitment of the $(UvrA)_2(UvrB)_1$ complex to the site of damage (E), where nucleotide excision repair takes place (F). (*Adapted from Selby and Sancar[173] with permission.*)

However, superficially they suggest that in the absence of functional Pol I, one or more Uvr proteins are required for some essential aspect of cellular metabolism. Since the *uvrB*$^+$ gene contains a binding site for DnaA protein, it has been suggested that UvrB protein may be involved in DNA replication under some circumstances (46).

Detection and Measurement of Nucleotide Excision Repair

It is useful to know something about the commonly used experimental techniques for detecting and measuring biochemical events associated with nucleotide excision repair, particularly since many of these techniques are frequently referred to in the literature. The following section presents the essential principles of several

of these techniques, with a special emphasis on those used for studies with intact bacterial cells. As will be seen in later chapters, other techniques have lent themselves well to the qualitative and quantitative evaluation of nucleotide excision repair in eukaryotic cells.

EXCISION OF DAMAGED BASES

Loss of Radiolabeled Pyrimidine Dimers from DNA

In DNA radiolabeled in thymine (usually with ^{14}C or ^{3}H), excision of thymine-containing pyrimidine dimers can be monitored by demonstrating the transfer of radiolabeled dimers to an ethanol- or acid-soluble fraction, since high-molecular-weight DNA is readily precipitated by ethanol or acid. It is of course necessary to resolve the radiolabel specifically associated with dimers from that associated with nondimer nucleotides that are also transferred to the soluble phase after precipitation of DNA. These techniques have a number of important limitations that must be kept in mind. For one thing, it is very difficult to reliably detect the excision of thymine-containing pyrimidine dimers from DNA when these lesions represent a very small fraction of the total radioactivity in thymine (<0.05%), unless highly sensitive separation techniques (such as high-pressure liquid chromatography) are used. Second, the loss of dimers from high molecular weight DNA does not distinguish dimer excision catalyzed by a PD-DNA glycosylase/AP endonuclease from that catalyzed by a UvrABC-type of endonuclease. Hence, the simple demonstration of the transfer of thymine dimers from the insoluble to the soluble fraction has no specific mechanistic implications.

Loss of DNA Sites Sensitive to Specific Enzyme Probes

The *M. luteus* and phage T4 PD-DNA glycosylases are absolutely specific for pyrimidine dimers in DNA (see chapter 4). If these lesions are removed during nucleotide excision repair and the covalent integrity of the DNA is restored by repair synthesis and DNA ligation, DNA isolated from such cells will obviously no longer be sensitive to incision by these enzymes. Thus, when such DNA is incubated with one of these DNA glycosylases and then denatured and sedimented in alkaline sucrose gradients (or treated in some other way that allows the measurement of the molecular weight of single-stranded DNA), it will have a higher single-stranded molecular weight than will DNA in which dimers are still present (Fig. 5–18) (51, 133). This assay of nucleotide excision repair is sometimes called the *loss of enzyme (or endonuclease-) sensitive site assay.*

This general technique is quantitatively more sensitive than the direct measurement of the loss of thymine-containing pyrimidine dimers from DNA and is particularly well suited to studies in which cells are exposed to very low levels of UV radiation. Furthermore, the loss of enzyme-sensitive sites measures the excision of all pyrimidine dimers and hence is not limited to those containing thymine, which of course is necessarily the case when pyrimidine dimers are tracked through radioactivity in thymine. In principle, this general technology is applicable to any lesion in DNA for which specific enzyme probes exist. However, like the measurement of the transfer of damaged bases to the acid- or ethanol-soluble fraction of DNA, it does not yield information about the precise mechanism of the excision repair.

The emergence of sensitive techniques for detecting specific DNA sequences by nucleic acid hybridization combined with the use of enzyme probes specific for particular types or classes of DNA damage has facilitated an extremely sensitive technique for measuring nucleotide excision repair of pyrimidine dimers and other forms of base damage in single-copy DNA sequences. This technique is discussed in detail in chapter 7.

Appearance and Disappearance of Strand Breaks in DNA

Damage-specific incision of DNA can be monitored by a variety of techniques which directly detect the strand breaks (or nicks) that are enzymatically produced in vivo, rather than detecting excision of the substrate lesions themselves (4, 18,

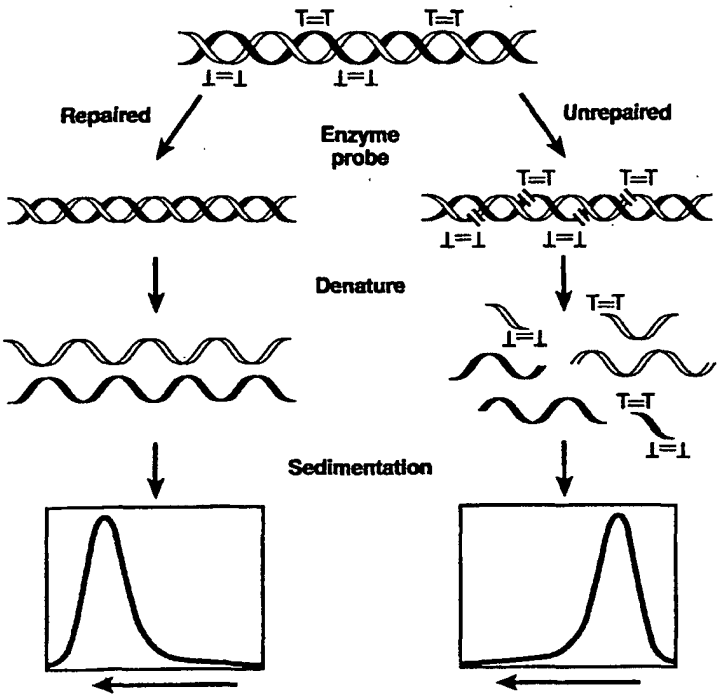


Figure 5–18 The presence of unrepaired pyrimidine dimers in the DNA of UV-irradiated cells can be detected with the use of dimer-specific enzyme probes such as the *M. luteus* or phage T4 PD-DNA glycosylase/AP lyases (see chapter 4). Radiolabeled DNA is extracted from cells and incubated with the enzyme. The enzyme catalyzes the formation of strand breaks at pyrimidine dimer (enzyme-sensitive) sites. DNA containing dimers (enzyme-sensitive sites) will sediment more slowly in alkaline sucrose gradients than will DNA containing no (or fewer) dimers. (*Adapted from Friedberg [45a] with permission.*)

32, 67, 83, 96, 160). When cells have undergone complete nucleotide excision repair and the covalent integrity of the DNA has been restored, these strand breaks obviously disappear. The disappearance of damage-specific strand breaks is therefore a useful indicator of nucleotide excision repair. Since this method does not require identification of the particular lesion being repaired, strand breaks that are spuriously introduced into DNA (and are thus unrelated to the repair of base damage) may not be readily distinguished from those that are related to repair. The extensively studied model of DNA containing pyrimidine dimers serves as an example of how this problem can be overcome in specific instances. Pyrimidine dimers are subject to repair by enzymatic photoreactivation (see chapter 2). One can therefore ask whether the strand breaks that appear during nucleotide excision repair fail to appear if the cells are subjected to photoreactivation prior to excision repair. If so, the breaks are presumably incisions at pyrimidine dimers. Alternatively, the specificity of DNA strand breaks observed in wild-type cells can be evaluated by direct comparison with mutants defective in incision of DNA at the lesions in question.

Quantitative PCR

Many alterations in DNA bases result in inhibition of the PCR catalyzed by *Taq* DNA polymerase. Hence, the presence of base damage can be detected by reduced PCR amplification when this is measured quantitatively. Since only nondamaged templates participate in PCR, this assay can accurately measure the fraction of template DNA molecules that contain no damage (26). Assuming a random

distribution of base damage, the Poisson equation allows the calculation of the average frequency of base damage per DNA strand (26).

Measurement of Repair Synthesis

The most general method used for measuring repair synthesis is by density labeling of the DNA (191) (Fig. 5–19). In this procedure, DNA synthesis following damage is carried out in the presence of bromo-dUTP. The incorporation of sufficient amounts of 5-bromouracil (5-BU) instead of thymine imparts an increased buoyant density to the DNA. Such increases are generally too small to be detectable in regions of nonsemiconservative (repair) synthesis because the incorporated 5-BU constitutes only a tiny fraction of the mass of the DNA fragments isolated by usual procedures. However, a significant increase in density can usually be detected in regions of semiconservative replication. Thus, with the use of radiolabeled 5-BU, DNA undergoing semiconservative synthesis can be separated from DNA exclusively undergoing repair synthesis by sedimentation in isopycnic gradients, because in the former case the 5-BU is typically incorporated into an entire

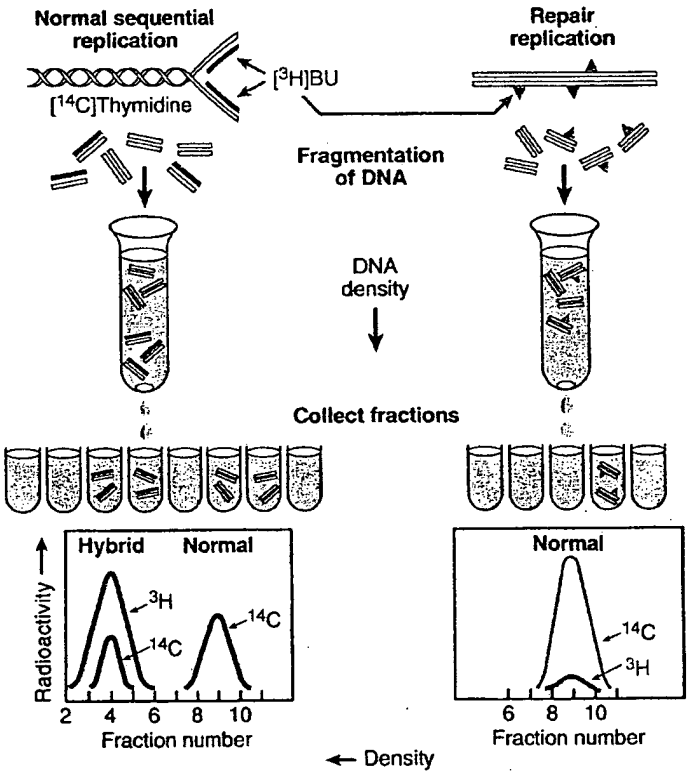


Figure 5–19 Schematic illustration of the detection of repair synthesis by buoyant density centrifugation of DNA containing 5-BU. DNA is prelabeled with [^{14}C]thymidine to provide a uniform label. Following exposure to UV radiation (or some other form of DNA damage), repair synthesis during nucleotide excision repair takes place in the presence of [^{3}H]BU. DNA synthesized both by semiconservative and by non-semiconservative modes will thus be density labeled (red). To distinguish these, the DNA is fragmented (by shearing) and sedimented to equilibrium density. Fragments of DNA-containing strands that were synthesized semiconservatively will have a hybrid density detected by the position of the ^{3}H radiolabel (left). Repair synthesis patches are too small to alter the density of the DNA, and hence the ^{3}H radiolabel appears at the position of normal density DNA (right). (*Adapted from Friedberg [45a] with permission.*)

strand of a given DNA fragment and hence constitutes an appreciable fraction of its mass. The radioactivity incorporated into unreplicated (parental density) DNA is then a measure of the total amount of repair synthesis (Fig. 5–19).

The accuracy of this measurement is influenced chiefly by the amount of background semiconservative DNA synthesis. Thus, wherever feasible, selective inhibition of the latter is attempted. In prokaryotes such as *E. coli*, such selective inhibition is difficult to achieve, although semiconservative replication is reduced considerably by DNA damage itself (196). Refinements of the density-labeling technique allow for estimates of the size of the regions (patches) of repair synthesis in DNA. Most simply, the amount of radiolabel incorporated during repair, together with an independent determination of the number of repair events, can be used to calculate the average repair patch size (36). An alternative procedure involves shearing the DNA to known small size by sonication of the isolated parental density fraction containing repair patches. The repair patches now constitute an appreciable fraction of the length of the DNA fragments. The DNA is then analyzed in alkaline isopycnic gradients so that the density shift of only the affected strands is measured. The observed increase in the density of the DNA fragments, together with the measured average size of the fragments, yields an estimate of the average size of the repair patches (192).

Another procedure for measuring the size of repair synthesis patches in DNA exploits the photolytic sensitivity of DNA containing 5-BU (177). When DNA containing this thymine analog is exposed to radiation at 313 nm, debromination followed by free radical attack of the deoxyribose or deoxyribose-phosphate backbone occurs, resulting in DNA strand breaks and/or formation of alkali-labile sites (177). Alkaline sucrose gradient sedimentation can then be used to measure the extent of DNA fragmentation (Fig. 5–20). If enough 313-nm UV radiation can be delivered to achieve a plateau level of fragmentation (i.e., produce at least one break or alkali-labile site per repair patch), the average patch size can be derived directly from the known efficiency of the 5-BU photolysis (177).

Nucleotide Excision Repair in Other Prokaryotes

M. LUTEUS

As indicated in chapter 4, *M. luteus* is endowed with a DNA-glycosylase/AP endonuclease (UV endonuclease) which specifically repairs pyrimidine dimers by a base excision repair mode. However, mutants that are defective in this enzymatic activity are not abnormally sensitive to UV radiation under nonphotoreactivating conditions (201), suggesting that this organism may possess alternative mechanisms for excising pyrimidine dimers from DNA. Consistent with this suggestion, highly UV-sensitive mutants were identified with normal levels of PD-DNA glycosylase activity (197, 201). Like the *uvr* mutants of *E. coli*, these mutants are abnormally sensitive to killing to other agents such as mitomycin and 4-nitroquinoline 1-oxide (59, 197, 201).

M. luteus genes homologous to the *uvrA*$^+$ and *uvrB*$^+$ genes of *E. coli* have been cloned (119, 183, 184). The *uvrA*$^+$ gene complements the multiple sensitivities of a *M. luteus* strain designated DB7, which is defective in *uvrA*. The cloned gene has an open reading frame of 992 codons [slightly longer than that of the *E. coli uvrA*$^+$ gene (940 codons)]. The *M. luteus uvrA*$^+$ gene shares extensive amino acid sequence homology with the translated sequence of its *E. coli* homolog. Additionally, all of the presumed functional elements revealed by the sequence of the *E. coli uvrA*$^+$ gene (nucleotide binding domains and zinc fingers) are conserved (119, 184). Similar homology exists between the *E. coli* and *M. luteus uvrB*$^+$ genes. The latter complements the phenotypes of a *uvrB* mutant strain designated UV^{S}N^I and has an open reading frame of 709 amino acids; again somewhat longer than the 672 codons in the *E. coli uvrB* open reading frame (119, 183). One would predict that a homolog of the *E. coli uvrC*$^+$ gene also exists in *M. luteus*. However, to date no mutants distinct from those defective in the *uvrA* and *uvrB* genes have been identified.

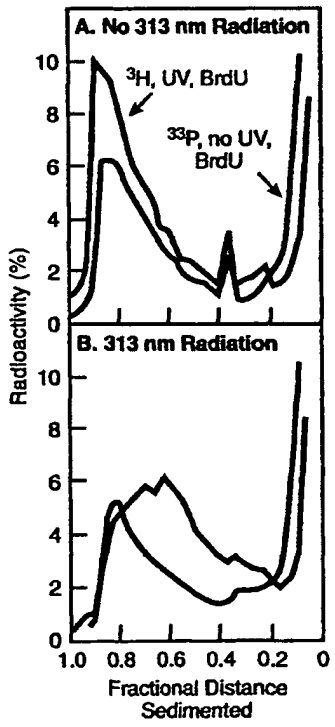


Figure 5–20 Sedimentation patterns in alkaline sucrose of labeled DNA from normal human fibroblasts treated or not treated with UV radiation and allowed to repair in the presence of 5-BU before exposure to 313-nm photolysis. Photolysis of incorporated 5-BU results in strand breakage of the DNA when sedimented in alkali (top). No degradation of DNA is observed in the absence of photolytic irradiation (bottom). The amount of 313-nm radiation required to cause strand breakage at all sites of 5-BU incorporation provides a means for estimating the size of the DNA synthesis (repair) patches. (*Adapted from Friedberg [45a] with permission.*)

DEINOCOCCUS RADIODURANS

Deinococcus (formerly *Micrococcus*) *radiodurans* and other members of the same genus are extremely resistant to the lethal and mutagenic effects of most agents that damage DNA, including UV radiation. Hence, this organism presents interesting opportunities for exploring the biochemistry and molecular biology of nucleotide excision repair in fully viable populations of cells that have sustained large numbers of "hits" in their genome. *D. radiodurans* lacks DNA photolyase activity (112). Nonetheless, the D_{37} for UV radiation exposure (the dose of UV radiation that yields 37% survival) is much higher than that observed with *E. coli* (~880 and <50 J/m², respectively) (65). There is nothing unusual about the efficiency of the formation of photoproducts in the DNA of *D. radiodurans* cells exposed to UV radiation, except that there is a higher relative ratio of (6-4) photoproducts to pyrimidine dimers (217), probably reflecting the high G+C content of the DNA of this organism, which results in many more TC dinucleotide sequences (116).

Within 90 min following exposure of *D. radiodurans* cells to UV radiation, the content of thymine-containing pyrimidine dimers is reduced from 1.7 to 0.3% (217). This extraordinarily efficient (presumably excision) repair of pyrimidine dimers was first demonstrated by Bolling and Setlow (16), who showed that the many pyrimidine dimers excised from the genome of *D. radiodurans* appear in the medium rather than in the acid-soluble fraction of DNA. It is now established that *D. radiodurans* is endowed with two independent excision repair pathways, either of which alone is sufficient to endow the organism with wild-type resistance to UV radiation at 254 nm. One of these pathways appears to operate by nucleotide excision and is believed to require at least two genes, originally designated *mtcA*⁺ and *mtcB*⁺ (5, 41, 113). In addition to pyrimidine dimers, this pathway can process mitomycin-induced DNA-DNA cross-links and bulky chemical adducts. The proteins encoded by the *mtcA*⁺ and *mtcB*⁺ genes have not yet been physically identified. However, it was proposed that these proteins are required for the activity of an endonuclease called UV endonuclease-α. It has since been determined that *mtcA* and *mtcB* are in fact portions of a single gene and that this gene has extensive amino acid sequence homology with *uvrA*⁺ of *E. coli* and *M. luteus* (1). Hence, *mtcA* and *mtcB* have been renamed *uvrA1* and *uvrA2*, respectively, and the full-length wild-type allele is designated *uvrA*⁺, to conform with the *E. coli* nomenclature for nucleotide excision repair genes. The UvrA protein of *D. radiodurans* presumably participates in a pathway formally analogous to that of the UvrABC-mediated pathway of *E. coli*. However, to date *uvrB*⁺ and *uvrC*⁺ homologs have not been identified in *D. radiodurans*.

In contrast to the situation in *E. coli*, *uvrA* mutants of *D. radiodurans* retain essentially wild-type levels of resistance to UV radiation and are able to remove pyrimidine dimers from their DNA by the action of a second endonuclease called UV endonuclease-β, which requires the gene(s) *uvsCDE*⁺. To date, no other substrates have been demonstrated for this endonuclease activity. Mutants defective in both endonucleases (e.g., *uvrA1 uvsE* double mutants) are highly sensitive to killing by UV radiation and are defective in the incision of UV-irradiated DNA (41, 113).

The limited substrate specificity of UV endonuclease-β has led to the suggestion that it is a PD-DNA glycosylase, analogous to the phage T4 and *M. luteus* PD-DNA glycosylases discussed in chapter 4. UV endonuclease-β has been partially purified and found to have a molecular mass of ~36 kDa. The endonuclease activity has a novel requirement for Mn^{2+} ions (42), and, in contrast to the T4 and *M. luteus* PD-DNA glycosylases, it is inactive in the presence of EDTA. Furthermore, its mode of action appears to be directly endonucleolytic rather than glycosylic, since incisions in DNA catalyzed by the enzyme do not generate free thymine following photoreversal (43) (see chapter 4). In addition to these distinguishing features, the *D. radiodurans* UV endonuclease-β activity confers wild-type levels of resistance to UV radiation in *uvrA* mutants. In contrast, in *M. luteus* the UV sensitivity of *uvrA* mutants is only marginally corrected by introduction of the gene

which encodes the endogenous PD-DNA glycosylase (120). These and other similar observations (66) have led to the speculation that UV endonuclease-β might also recognize (6-4) photoproducts in DNA, which account for as much as 17% of the thymine-containing bimolecular photoproducts in the DNA of *D. radiodurans* cells exposed to UV radiation. The definitive conclusion that UV endonuclease-β is a true direct-acting repair endonuclease, as distinct from a PD-DNA glycosylase, must await more-intensive study of this interesting enzyme.

OTHER ORGANISMS

In several other prokaryotes, including *Salmonella typhimurium, Pseudomonas fluorescens, Streptococcus pneumoniae, Bacillus subtilis,* and *Mycoplasma genitalium,* pyrimidine dimers are excised as oligonucleotide fragments of 13 nucleotides by hydrolysis of the eighth phosphodiester bond 5′ to the dimer and the fifth phosphodiester bond 3′ to the lesion (28). Hence, it seems reasonable to conclude that the mechanism of nucleotide excision repair so elegantly deciphered in *E. coli* is conserved in prokaryotes.

Other Damage-Specific DNA-Incising Activities?

ENDONUCLEASE V OF *E. COLI*

A small protein that degrades a variety of substrates which share no obvious structural similarity has been purified from *E. coli* and is termed *endonuclease V* (39, 52). This enzyme has an absolute requirement for $MgCl_2$ for activity. Among the substrates for the purified enzyme are duplex UV-irradiated DNA (at sites other than pyrimidine dimers), DNA treated with osmium tetroxide, heat- or acid-depurinated DNA, and DNA containing adducts of 7-bromomethylbenz[*a*]anthracene (39, 52). In addition, native DNA from phage PBS2 (a DNA that contains uracil instead of thymine [see chapter 1]) and phage T5 DNA containing thymine substituted by uracil are degraded by the enzyme (39, 52). The enzyme also attacks the single-stranded DNA from phage fd and duplex fd RFI DNA (39, 52).

It is difficult to identify a common determinant in all of these substrates, and the basis for the apparent broad substrate specificity of endonuclease V remains unclear. An interesting possibility is that the enzyme is single strand specific and therefore recognizes single-stranded areas in duplex DNA created by relatively nonspecific base damage. Endonuclease V is optimally active at pH 9.5 and has little if any detectable activity at neutral pH. The alkaline pH optimum might facilitate helix destabilization in areas of localized conformational distortion in duplex DNA. This could explain the observed degradation of PBS2 DNA, since at pH 9.5 the disruption of A·U base pairs may occur more readily than that of A·T base pairs.

When endonuclease V reactions with various DNAs are sedimented in alkali to monitor single-strand breaks (nicks) and in neutral sucrose to measure double strand breaks, the ratio of single-strand nicks to double-strand breaks is only 8:1 over a wide range of nicks and a sixfold range of enzyme concentration (39). Thus, the double-strand breaks apparently do not arise from the random juxtaposition of two nicks or from an enzyme concentration-dependent alteration in the mechanism of endonucleolytic cleavage of DNA (39).

Endonuclease V is present at normal levels in all *E. coli* mutants examined thus far, including a number of strains defective in functions required for replication, recombination, and repair. Although the multiplicity of substrates recognized by this activity might be interpreted in terms of its role in DNA repair, the determination of the true cellular function of endonuclease V must await the isolation of mutants defective in the activity (39).

Summary and Conclusions

Damage-specific endonucleases such as the UvrABC enzyme of *E. coli* and the products of certain *RAD* gene products in *S. cerevisiae* and of the genes in human cells that are defective in human patients with the disease xeroderma pigmento-

sum (see later chapters) are singularly important enzymes for excision repair. Cellular and biological studies with mutants defective in the genes encoding these products suggest that these endonucleases are required for the incision of DNA containing a large spectrum of base damage. Thus, it is probable that the nucleotide excision repair pathway initiated by these damage-specific enzymes is a much more general mode for excision repair of base damage than is the base excision repair mechanism in which limited types of base damage are excised by specific DNA glycosylases.

It is noteworthy that some types of base damage, including sites of base loss generated by DNA glycosylases, are recognized by both the base and nucleotide excision repair pathways, allowing for possible convergence of these two general pathways of excision repair (193). Such convergence may exclude repair by one pathway in the presence of the other, leading to unexpected phenotypes for certain mutants. For example, the binding of the $(UvrA)_2(UvrB)_1$ complex to AP sites can inhibit base excision repair of such lesions (193). If the completion of the repair of AP sites by nucleotide excision repair was inhibited by a mutant *uvrC* gene, it is possible that such mutant strains would confer abnormal sensitivity to types of damage typically repaired by the base excision repair pathway.

The availability of highly purified gene products encoded by genes cloned into recombinant DNA molecules has unquestionably overcome the quantitative problems that hampered progress in this area of nucleic acid enzymology for so long. Despite the impressive progress made with *E. coli*, much remains to be learned about the details of the biochemistry of nucleotide excision repair in this organism and particularly in other prokaryotes.

REFERENCES

1. **Agostini, H., and K. W. Minton.** 1994. Personal communication.

2. **Ahmed, S. I., and I. B. Holland.** 1985. Isolation and analysis of a mutant of *Escherichia coli* hyper-resistant to near-ultraviolet light plus 8-methoxypsoralen. *Mutat. Res.* **151**:43–47.

3. **Ahn, B. C., and L. Grossman.** 1994. Personal communication.

4. **Ahnström, G., and K. Erixon.** 1981. Measurement of strand breaks by alkaline denaturation and hydroxyapatite chromatography, p. 403–418. *In* E. C. Friedberg and P. C. Hanawalt (ed.), *DNA Repair—A Laboratory Manual of Research Procedures*, vol. 1, part A. Marcel Dekker, Inc., New York.

5. **Al-Bakri, G. H., M. W. Mackay, P. A. Whittaker, and B. E. B. Moseley.** 1985. Cloning of the DNA repair genes *mtcA, mtcB, uvsC, uvsE* and the *leuB* gene from *Deinococcus radiodurans*. *Gene* **33**:305–311.

6. **Arikan, E., M. S. Kulkarni, D. C. Thomas, and A. Sancar.** 1986. Sequences of the *E. coli uvrB* gene and protein. *Nucleic Acids Res.* **14**:2637–2650.

7. **Arthur, H. M., D. Bramhill, P. B. Eastlake, and P. T. Emmerson.** 1982. Cloning of the *uvrD* gene of *E. coli* and identification of the product. *Gene* **19**:285–295.

8. **Backendorf, C., H. Spaink, A. P. Barbeiro, and P. Van de Putte.** 1986. Structure of the *uvrB* gene of *Escherichia coli*. Homology with other DNA repair enzymes and characterization of the *uvrB5* mutation. *Nucleic Acids Res.* **14**:2877–2890.

9. **Backendorf, C. M., E. A. Van den Berg, J. A. Brandsma, T. Kartasova, C. A. Van Sluis, and P. Van de Putte.** 1983. In vivo regulation of the *uvr* and *ssb* genes in *Escherichia coli*, p. 161–171. *In* E. C. Friedberg and B. A. Bridges (ed.), *Cellular Responses to DNA Damage*. Alan R. Liss, Inc., New York.

10. **Beck, D. J., S. Popoff, A. Sancar, and W. D. Rupp.** 1985. Reactions of the UVRABC excision nuclease with DNA damaged by diamminedichloroplatinum(II). *Nucleic Acids Res.* **13**:7395–7412.

11. **Ben-Ishai, R., and R. Sharon.** 1981. On the nature of the repair deficiency in *E. coli uvrE*, p. 147–151. *In* E. Seeberg and K. Kleppe (ed.), *Chromosome Damage and Repair*. Plenum Publishing Corp, New York.

12. **Berg, J. M.** 1986. Potential metal-binding domains in nucleic acid binding proteins. *Science* **232**:485–487.

13. **Bertrand-Burggraf, E., S. Hurstel, M. Daune, and M. Schnarr.** 1987. Promoter properties and negative regulation of the *uvrA* gene by the LexA repressor and its amino-terminal DNA binding domain. *J. Mol. Biol.* **193**:293–302.

14. **Bertrand-Burggraf, E., C. P. Selby, J. E. Hearst, and A. Sancar.** 1991. Identification of the different intermediates in the interaction of (A)BC excinuclease with its substrates by DNase I footprinting on two uniquely modified oligonucleotides. *J. Mol. Biol.* **218**:27–36.

15. **Bockrath, R. C., and J. E. Palmer.** 1977. Differential repair of premutational UV-lesions at tRNA genes in *E. coli*. *Mol. Gen. Genet.* **156**:133–140.

16. **Bolling, M. E., and J. K. Setlow.** 1966. The resistance of *Micrococcus radiodurans* to ultraviolet radiation a repair mechanism. *Biochim. Biopys. Acta.* **123**:26–33.

17. **Boyce, R. P., and P. Howard-Flanders.** 1964. Release of ultraviolet light-induced thymine dimers from DNA in *E. coli* K-12. *Proc. Natl. Acad. Sci. USA* **51**:293–300.

18. **Braun, A. G.** 1981. Measurement of strand breaks by nitrocellulose membrane filtration, p. 447–455. *In* E. C. Friedberg and P. C. Hanawalt (ed.), *DNA Repair—A Laboratory Manual of Research Procedures*, vol. 1, part B. Marcel Dekker, Inc., New York.

19. **Bridges, B. A.** 1983. Psoralens and serendipity: aspects of the genetic toxicology of 8-methoxypsoralen. *Environ. Mutagen.* **5**:329–339.

20. **Bridges, B. A.** 1984. Further characterization of repair of 8-methoxypsoralen crosslinks in UV-excision-defective *Escherichia coli*. *Mutat. Res.* **132**:153–160.

21. **Bridges, B. A., and M. Stannard.** 1982. A new pathway for repair of cross-linkable 8-methoxypsoralen mono-adducts in *uvr* strains of *Escherichia coli*. *Mutat. Res* **92**:9–14.

22. **Campbell, J. L., L. Soll, and C. C. Richardson.** 1972. Isolation and partial characterization of a mutant of *Escherichia coli* deficient in DNA polymerase II. *Proc. Natl. Acad. Sci. USA* **69**:2090–2094.

23. **Caron, P. R., and L. Grossman.** 1988. Involvement of a cryptic ATPase activity of UvrB and its proteolysis product, UvrB*, in DNA repair. *Nucleic Acids Res.* **16**:9651–9662.

24. **Caron, P. R., and L. Grossman.** 1988. Potential role of proteolysis in the control of UvrABC incision. *Nucleic Acids Res.* **16**:9641–9650.

25. **Caron, P. R., S. R. Kushner, and L. Grossman.** 1985. Involvement of helicase II (*uvrD* gene product) and DNA polymerase I in excision mediated by the uvrABC protein complex. *Proc. Natl. Acad. Sci. USA* **82**:4925–4929.

26. **Chandrasekhar, D., and B. Van Houten.** 1994. High resolution mapping of UV-induced photoproducts in the *Escherichia coli lacI* gene. Inefficient repair of the non-transcribed strand correlates with high mutation frequency. *J. Mol. Biol.* **238**:319–332.

27. **Cheng, S., A. Sancar, and J. E. Hearst.** 1991. RecA-dependent incision of psoralen-crosslinked DNA by (A)BC excinuclease. *Nucleic Acids Res.* **19**:657–663.

28. **Cheng, S., B. Van Houten, H. B. Gamper, A. Sancar, and J. E. Hearst.** 1988. Use of psoralen-modified oligonucleotides to trap three-stranded RecA-DNA complexes and repair of these cross-linked complexes by ABC excinuclease. *J. Biol. Chem.* **263**:15110.

29. **Cheng, S., B. Van Houten, H. B. Gamper, A. Sancar, and J. E. Hearst.** 1988. Use of triple-stranded DNA complexes to study crosslink repair, p. 105–113. *In* E. C. Friedberg and P. C. Hanawalt (ed.), *Mechanisms and Consequences of DNA Damage Processing*. Alan R. Liss, Inc., New York.

30. **Claassen, L. A., B. Ahn, H. S. Koo, and L. Grossman.** 1991. Construction of deletion mutants of the *Escherichia coli* UvrA protein and their purification from inclusion bodies. *J. Biol. Chem.* **266**:11380–11387.

31. **Claassen, L. A., and L. Grossman.** 1991. Deletion mutagenesis of the *Escherichia coli* UvrA protein localizes domains for DNA binding, damage recognition, and protein-protein interactions. *J. Biol. Chem.* **266**:11388–11394.

32. **Clayton, D. A.** 1981. Measurement of strand breaks in supercoiled DNA by electron microscopy, p. 419–424. *In* E. C. Friedberg and P. C. Hanawalt (ed.), *DNA repair—A Laboratory Manual of Research Procedures*, vol. 1, part B. Marcel Dekker, Inc., New York.

33. **Cole, R. S.** 1973. Repair of DNA containing interstrand crosslinks in *Escherichia coli*: sequential excision and recombination. *Proc. Natl. Acad. Sci. USA* **70**:1064–1068.

34. **Cooper, P. K.** 1977. Excision-repair in mutants of *Escherichia coli* deficient in DNA polymerase I and/or its associated 5′ → 3′ exonuclease. *Mol. Gen. Genet.* **150**:1–12.

35. **Cooper, P. K.** 1982. Characterization of long patch excision repair of DNA in ultraviolet-irradiated *Escherichia coli*: an inducible function under *rec-lex* control. *Mol. Gen. Genet.* **185**:189–197.

36. **Cooper, P. K., and P. C. Hanawalt.** 1972. Heterogeneity of patch size in repair replicated DNA in *Escherichia coli*. *J. Mol. Biol.* **67**:1–10.

37. **Cooper, P. K., and P. C. Hanawalt.** 1972. Role of DNA polymerase I and the *rec* system in excision-repair in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **69**:1156–1160.

38. **Cooper, P. K., and J. G. Hunt.** 1978. Alternative pathways for excision and resynthesis in *Escherichia coli*: DNA polymerase III role? p. 255–260. *In* P. C. Hanawalt, E. C. Friedberg, and C. F. Fox (ed.), *DNA Repair Mechanisms*. Academic Press, Inc., New York.

39. **Demple, B., and S. Linn.** 1982. On the recognition and cleavage mechanism of *Escherichia coli* endodeoxyribonuclease V, a possible DNA repair enzyme. *J. Biol. Chem.* **257**:2848–2855.

40. **Doolittle, R. F., M. S. Johnson, I. Husain, B. Van Houten, D. C. Thomas, and A. Sancar.** 1986. Domainal evolution of a prokaryotic DNA repair protein and its relationship to active-transport proteins. *Nature* (London) **323**:451–453.

41. **Evans, D. M., and B. E. B. Moseley.** 1983. Roles of *uvsC*, *uvsD*, *uvsE*, and *mtcA* genes in the two pyrimidine dimer excision repair pathways of *Deinococcus radiodurans*. *J. Bacteriol.* **156**:576–583.

42. **Evans, D. M., and B. E. B. Moseley.** 1985. Identification and initial characterization of a pyrimidine dimer endonuclease (UV endonuclease b) from *Deinococcus radiodurans*; a DNA-repair enzyme that requires manganese ions. *Mutat. Res.* **145**:119–128.

43. **Evans, D. M., and B. E. B. Moseley.** 1988. *Deinococcus radiodurans* UV endonuclease β DNA incisions do not generate photoreversible thymine residues. *Mutat. Res.* **207**:117–119.

44. **Fogliano, M., and P. F. Schendel.** 1981. Evidence for the inducibility of the *uvrB* operon. *Nature* (London) **289**:196–198.

45. **Forster, J. W., and P. Strike.** 1985. Organization and control of the *Escherichia coli uvrC* gene. *Gene* **35**:71–82.

45a. **Friedberg, E. C.** 1985. *DNA Repair*. W. H. Freeman Co., New York.

46. **Friedberg, E. C.** 1987. The molecular biology of nucleotide excision repair of DNA: recent progress. *J. Cell Sci. Suppl.* **6**:1–23.

47. **Friedberg, E. C., and B. A. Bridges.** 1983. *Cellular Responses to DNA Damage*. Alan R. Liss, Inc., New York.

48. **Friedberg, E. C., and P. C. Hanawalt.** 1981. *DNA Repair—A Laboratory Manual of Research Procedures*, vol. 1, Part A. Marcel Dekker, Inc., New York.

49. **Friedberg, E. C., and P. C. Hanawalt.** 1981. *DNA Repair—A Laboratory Manual of Research Procedures*, vol. 2. Marcel Dekker, Inc., New York.

50. **Fuller, R. S., B. E. Funnell, and A. Kornberg.** 1984. The dnaA protein complex with the *E. coli* chromosomal replication origin (oriC) and other DNA sites. *Cell* **38**:889–900.

51. **Ganesan, A. K., C. A. Smith, and A. A. Van Zeeland.** 1981. Measurement of the pyrimidine dimer content of DNA in permeabilized bacterial or mammalian cells with endonuclease V of bacteriophage T4, p. 89–97. *In* E. C. Friedberg and P. C. Hanawalt (ed.), *DNA Repair—A Laboratory Manual of Research Procedures*, vol. 1, part A. Marcel Dekker, Inc., New York.

52. **Gates, F. T., III, and S. Linn.** 1977. Endonuclease V of *Escherichia coli*. *J. Biol. Chem.* **252**:1647–1653.

53. **George, D. L., and E. M. Witkin.** 1975. Ultraviolet light-induced responses of an *mfd* mutant of *Escherichia coli* B/r having a slow rate of dimer excision. *Mutat. Res.* **28**:347–354.

54. **Gopalakrishnan, A. S., Y. C. Chen, M. Temkin, and W. Dowhan.** 1986. Structure and expression of the gene locus encoding the phosphatidylglycerophosphate synthase of *Escherichia coli*. *J. Biol. Chem.* **261**:1329–1338.

55. **Gorbalenya, A. E., and E. V. Koonin.** 1990. Superfamily of UvrA-related NTP-binding proteins. Implications for rational classification of recombination/repair systems. *J. Mol. Biol.* **213**:583–591.

56. **Granger-Schnarr, M., M. Schnarr, and C. A. Van Sluis.** 1986. In vitro study of the interaction of the LexA repressor and the UvrC protein with a *uvrC* regulatory region. *FEBS Lett.* **198**:61–65.

57. **Gross, J., and M. Gross.** 1969. Genetic analysis of an *E. coli* strain with a mutation affecting DNA polymerase. *Nature* (London) **224**:1166–1168.

58. **Grossman, L., P. R. Caron, S. J. Mazur, and E. Y. Oh.** 1988. Repair of DNA-containing pyrimidine dimers. *FASEB J.* **2**:2696–2701.

59. **Grossman, L., J. C. Kaplan, S. R. Kushner, and I. Mahler.** 1967. Enzymes involved in the early stages of repair of ultraviolet-irradiated DNA. *Cold Spring Harbor Symp. Quant. Biol.* **33**:229–234.

60. **Grossman, L., S. J. Mazur, P. R. Caron, and E. Y. Oh.** 1988. Dynamics of the *E. coli uvr* DNA repair system, p. 73–77. *In* E. C. Friedberg and P. C. Hanawalt (ed.), *Mechanisms and Consequences of DNA Damage Processing*. Alan R. Liss, Inc., New York.

61. **Grossman, L., E. Y. Oh, S. Mazur, and P. Caron.** 1989. Macromolecular physiology of the *Escherichia coli* Uvr proteins, p. 11–15. *In* A. Castellani (ed.), *DNA Damage and Repair*. Plenum Publishing Corp., New York.

62. **Grossman, L., and S. Thiagalingam.** 1993. Nucleotide excision repair, a tracking mechanism in search of damage. *J. Biol. Chem.* **268**:16871–16874.

63. Grossman, L., and A. T. Yeung. 1990. The UvrABC endonuclease of *Escherichia coli*. *Photochem. Photobiol.* **51**:749–755.

64. Grossman, L., and A. T. Yeung. 1990. The UvrABC endonuclease system of *Escherichia coli*—a view from Baltimore. *Mutation Res.* **236**:213–221.

65. Gutman, P. D., P. Fuchs, L. Ouyang, and K. W. Minton. 1993. Identification, sequencing and targeted mutagenesis of a DNA polymerase gene required for the extreme radioresistance of *Deinococcus radiodurans*. *J. Bacteriol.* **175**:3581–3590.

66. Gutman, P. D., H. L. Yao, and K. W. Minton. 1991. Partial complementation of the UV sensitivity of *Deinococcus radiodurans* excision repair mutants by the cloned *denV* gene of bacteriophage T4. *Mutat. Res.* **254**:207–215.

67. Hagen, U. F. W. 1981. Measurement of strand breaks by end labeling. p. 431–445. *In* E. C. Friedberg and P. C. Hanawalt (ed.), *DNA Repair—A Laboratory Manual of Research Procedures*, vol. 1, part B. Marcel Dekker, Inc., New York.

68. Hanawalt, P. C. 1982. Perspectives on DNA repair and inducible recovery phenomena. *Biochimie* **64**:847–851.

69. Hanawalt, P. C., P. K. Cooper, A. K. Ganesan, and C. A. Smith. 1979. DNA repair in bacteria and mammalian cells. *Annu. Rev. Biochem.* **48**:783–836.

70. Henderson, T. A., P. M. Dombrosky, and K. D. Young. 1994. Artifactual processing of penicillin-binding proteins 7 and 1b by the OmpT protease of *Escherichia coli*. *J. Bacteriol.* **176**:256–259.

71. Higgins, C. F., I. D. Hiles, G. P. Salmond, D. R. Gill, J. A. Downie, I. J. Evans, I. B. Holland, L. Gray, S. D. Buckel, and A. W. Bell. 1986. A family of related ATP-binding subunits coupled to many distinct biological processes in bacteria. *Nature* (London) **323**:448–450.

72. Horii, Z. I., and A. J. Clark. 1973. Genetic analysis of the *recF* pathway to genetic recombination in *Escherichia coli* K12: isolation and characterization of mutants. *J. Mol. Biol.* **80**:327–344.

73. Howard-Flanders, P., and R. P. Boyce. 1966. DNA repair and genetic recombination: studies on mutants of *Escherichia coli* defective in these processes. *Radiat. Res.* **6**(Suppl.):156–184.

74. Howard-Flanders, P., R. P. Boyce, and L. Theriot. 1966. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. *Genetics* **53**:1119–1136.

75. Husain, I., J. Griffith, and A. Sancar. 1988. Thymine dimers bend DNA. *Proc. Natl. Acad. Sci. USA* **85**:2558–2562.

76. Husain, I., G. B. Sancar, S. R. Holbrook, and A. Sancar. 1987. Mechanism of damage recognition by *Escherichia coli* DNA photolyase. *J. Biol. Chem.* **262**:13188–13197.

77. Husain, I., B. Van Houten, D. C. Thomas, M. Abdel-Monem, and A. Sancar. 1985. Effect of DNA polymerase I and DNA helicase II on the turnover rate of UvrABC excision nuclease. *Proc. Natl. Acad. Sci. USA* **82**:6774–6778.

78. Husain, I., B. Van Houten, D. C. Thomas, and A. Sancar. 1986. Sequences of *Escherichia coli uvrA* gene and protein reveal two potential ATP binding sites. *J. Biol. Chem.* **261**:4895–4901.

79. Jones, B. K., and A. T. Yeung. 1988. Repair of 4,5′,8-trimethylpsoralen monoadducts and cross-links by the *Escherichia coli* UvrABC endonuclease. *Proc. Natl. Acad. Sci. USA* **85**:8410–8414.

80. Jones, B. K., and A. T. Yeung. 1990. DNA base composition determines the specificity of UvrABC endonuclease incision of a psoralen cross-link. *J. Biol. Chem.* **265**:3489–3496.

81. Kacinski, B. M., and W. D. Rupp. 1982. *E. coli* uvrB protein binds to DNA in the presence of uvrA protein. *Nature* (London) **294**:480–481.

82. Kacinski, B. M., A. Sancar, and W. D. Rupp. 1981. A general approach for purifying proteins encoded by cloned genes without using a functional assay: isolation of the *uvrA* gene product from radiolabeled maxicells. *Nucleic Acids Res.* **9**:4495–4508.

83. Kohn, K. W., R. A. G. Ewig, L. C. Erickson, and L. A. Zwelling. 1981. Measurement of strand breaks and cross-links by alkaline elution, p. 379–401. *In* E. C. Friedberg and P. C. Hanawalt (ed.), *DNA Repair—A Laboratory Manual of Research Procedures*, vol. 1, part B. Marcel Dekker, Inc., New York.

84. Kow, Y. W., S. S. Wallace, and B. Van Houten. 1990. UvrABC nuclease complex repairs thymine glycol, an oxidative DNA base damage. *Mutat. Res.* **235**:147–156.

85. Kozelka, J., G. A. Petsko, G. J. Quigley, and S. J. Lippard. 1986. High-salt and low-salt models for kinked adducts of cis-diamminedichloroplatinum (II) with oligonucleotide duplexes. *Inorg. Chem.* **25**:1075–1077.

86. Kuemmerle, N., R. Ley, and W. Masker. 1981. Analysis of resynthesis tracts in repaired *Escherichia coli* deoxyribonucleic acid. *J. Bacteriol.* **147**:333–339.

87. Kuemmerle, N. B., and W. E. Masker. 1980. Effect of the *uvrD* mutation on excision repair. *J. Bacteriol.* **142**:535–546.

88. Kumura, K., K. Oeda, M. Akiyama, T. Horiuchi, and M. Sekiguchi. 1983. The *uvrD* gene of *E. coli*: molecular cloning and expression, p. 51–62. *In* E. C. Friedberg and B. A. Bridges (ed.), *Cellular Responses to DNA Damage*. Alan R. Liss, Inc., New York.

89. Kumura, K., M. Sekiguchi, A. L. Steinum, and E. Seeberg. 1985. Stimulation of the UvrABC enzyme-catalyzed repair reactions by the UvrD protein (DNA helicase II). *Nucleic Acids Res.* **13**:1483–1492.

90. Kunala, S., and D. E. Brash. 1992. Excision repair at individual bases of the *Escherichia coli lacI* gene: relation to mutation hot spots and transcription coupling activity. *Proc. Natl. Acad. Sci. USA* **89**:11031–11035.

91. Kushner, S. R., V. F. Maples, A. Easton, L. Farrance, and P. Peramachi. 1983. Physical, biochemical, and genetic characterization of the *uvrD* gene, p. 153–159. *In* E. C. Friedberg and B. A. Bridges (ed.), *Cellular Responses to DNA Damage*. Alan R. Liss, Inc., New York.

92. Kushner, S. R., J. Shepherd, G. Edwards, and V. F. Maples. 1978. *uvrD, uvrE,* and *recL* represent a single gene, p. 251–254. *In* P. C. Hanawalt, E. C. Friedberg, and C. F. Fox (ed.), *DNA Repair Mechanisms*. Academic Press, Inc., New York.

93. Lambert, B., B. K. Jones, B. P. Roques, J. B. Le Pecq, and A. T. Yeung. 1989. The noncovalent complex between DNA and the bifunctional intercalator ditercalinium is a substrate for the UvrABC endonuclease of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **86**:6557–6561.

94. Lambert, B., B. P. Roques, and J. B. Le Pecq. 1988. Induction of an abortive and futile DNA repair process in *E. coli* by the antitumor DNA bifunctional intercalator, ditercalinium: role of *polA* in death induction. *Nucleic Acids Res.* **16**:1063–1078.

95. Lehmann, A. R., and P. Karran. 1981. DNA repair. *Int. Rev. Cytol.* **72**:101–146.

96. Lett, J. T. 1981. Measurement of single-strand breaks by sedimentation in alkaline sucrose gradients, p. 363–378. *In* E. C. Friedberg and P. C. Hanawalt (ed.), *DNA Repair—A Laboratory Manual of Research Procedures*, vol. 1, part B. Marcel Dekker, Inc., New York.

97. Lin, J. J., A. M. Phillips, J. E. Hearst, and A. Sancar. 1992. Active site of (A)BC excinuclease. II. Binding, bending, and catalysis mutants of UvrB reveal a direct role in 3′ and an indirect role in 5′ incision. *J. Biol. Chem.* **267**:17693–17700.

98. Lin, J. J., and A. Sancar. 1989. A new mechanism for repairing oxidative damage to DNA: (A)BC excinuclease removes AP sites and thymine glycols from DNA. *Biochemistry* **28**:7979–7984.

99. Lin, J. J., and A. Sancar. 1990. Reconstitution of nucleotide excision nuclease with UvrA and UvrB proteins from *Escherichia coli* and UvrC protein from *Bacillus subtilis*. *J. Biol. Chem.* **265**:21337–21341.

100. Lin, J. J., and A. Sancar. 1991. The C-terminal half of UvrC protein is sufficient to reconstitute (A)BC excinuclease. *Proc. Natl. Acad. Sci. USA* **88**:6824–6828.

101. Lin, J. J., and A. Sancar. 1992. Active site of (A)BC excinuclease. I. Evidence for 5′ incision by UvrC through a catalytic site involving Asp399, Asp438, Asp466, and His538 residues. *J. Biol. Chem.* **267**:17688–17692.

102. Lin, J. J., and A. Sancar. 1992. (A)BC excinuclease: the *Escherichia coli* nucleotide excision repair enzyme. *Mol. Microbiol.* **6**:2219–2224.

103. Maples, V. F., and S. R. Kushner. 1982. DNA repair in *Escherichia coli*: identification of the *uvrD* gene product. *Proc. Natl. Acad. Sci. USA* **79**:5616–5620.

104. Masker, W., P. C. Hanawalt, and H. Shizuya. 1973. Role of DNA polymerase II in repair replication in *Escherichia coli*. *Nature* (London) *New Biol.* **244**:242–243.

105. Mattern, I. E., M. P. Van Winden, and A. Rörsch. 1965. The range of action of genes controlling radiation sensitivity in *Escherichia coli*. *Mutat. Res.* **2**:111–131.

106. Matthews, B. W., H. Nicholson, and W. J. Becktel. 1987. Enhanced protein thermostability from site-directed mutations that decrease the entropy of unfolding. *Proc. Natl. Acad. Sci. USA* **84**:6663–6667.

107. Mazur, S., and L. Grossman. 1991. Dimerization of *Escherichia coli* UvrA and its binding to undamaged and ultraviolet light damaged DNA. *Biochemistry* **30**:4432–4443.

108. Mellon, I., and P. C. Hanawalt. 1989. Induction of the *Escherichia coli* lactose operon selectively increases repair of its transcribed DNA strand. *Nature* (London) **342**:95–98.

109. Michalke, H., and H. Bremer. 1969. RNA synthesis in *Escherichia coli* after irradiation with ultraviolet light. *J. Mol. Biol.* **41**:1–23.

110. Moolenaar, G. F., C. A. Van Sluis, C. Backendorf, and P. Van de Putte. 1987. Regulation of the *Escherichia coli* excision repair gene *uvrC*. Overlap between the *uvrC* structural gene and the region coding for a 24 kD protein. *Nucleic Acids Res.* **15**:4273–4289.

111. Morimyo, M., and Y. Shimazu. 1976. Evidence that the gene *uvrB* is indispensable for a polymerase I deficient strain of *Escherichia coli* K-12. *Mol. Gen. Genet.* **147**:243–250.

112. Moseley, B. E. B. 1983. Photobiology and radiobiology of *Micrococcus* (*Deinococcus*) *radiodurans*. *Photochem. Photobiol. Rev.* **7**:223–274.

113. Moseley, B. E. B., and D. M. Evans. 1983. Isolation and properties of strains of *Micrococcus* (*Deinococcus*) *radiodurans* unable to excise ultraviolet light-induced pyrimidine dimers from DNA: evidence for two excision pathways. *J. Gen. Microbiol.* **129**:2437–2445.

114. Munn, M. M., and W. D. Rupp. 1991. Interaction of the UvrABC endonuclease with DNA containing a psoralen monoadduct or cross-link. Differential effects of superhelical density and comparison of preincision complexes. *J. Biol. Chem.* **36**:24748–24756.

115. Murray, M. L. 1979. Substrate-specificity of *uvr* excision repair. *Environ. Mutagen.* **1**:347–352.

116. Murray, R. G. E. 1992. The family *Deinococcaceae*, p. 3732–3744. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), *The Prokaryotes*. 2nd ed. Springer-Verlag, New York.

117. Myles, G. M., and A. Sancar. 1991. Isolation and characterization of functional domains of UvrA. *Biochemistry* **30**:3834–3840.

118. Myles, G. M., B. Van Houten, and A. Sancar. 1987. Utilization of DNA photolyase, pyrimidine dimer endonucleases, and alkali hydrolysis in the analysis of aberrant ABC excinuclease incisions adjacent to UV-induced DNA photoproducts. *Nucleic Acids Res.* **15**:1227–1243.

119. Nakayama, H., and S. Shiota. 1988. Excision repair in *Micrococcus luteus*: evidence for a UvrABC homolog, p. 115–120. *In* E. C. Friedberg and P. C. Hanawalt (ed.), *Mechanisms and Consequences of DNA Damage Processing*. Alan R. Liss, Inc., New York.

120. Nakayama, H., S. Shiota, and K. Umezu. 1992. UV endonuclease-mediated enhancement of UV survival in *Micrococcus luteus*. *Mutat. Res.* **273**:43–48.

121. Navaratnam, S., G. M. Myles, R. W. Strange, and A. Sancar. 1989. Evidence from extended X-ray absorption fine structure and site-specific mutagenesis for zinc fingers in UvrA protein of *Escherichia coli*. *J. Biol. Chem.* **264**:16067–16071.

122. Nonho, T., S. Noji, S. Taniguchi, and T. Saito. 1989. The *narX* and *narL* genes encoding the nitrate-sensing regulators of *Escherichia coli* are homologous to a family of prokaryotic two-component regulatory genes. *Nucleic Acids Res.* **17**:2947–2957.

123. Oeda, K., T. Horiuchi, and M. Sekiguchi. 1981. Molecular cloning of the *uvrD* gene of *Escherichia coli* that controls ultraviolet sensitivity and spontaneous mutation frequency. *Mol. Gen. Genet.* **184**:191–199.

124. Oeda, K., T. Horiuchi, and M. Sekiguchi. 1982. The *uvrD* gene of *E. coli* encodes a DNA-dependent ATPase. *Nature* (London) **298**:98–100.

125. Ogawa, H., K. Shimada, and J. Tomizawa. 1968. Studies on radiation-sensitive mutants of *E. coli*. I. Mutants defective in the repair synthesis. *Mol. Gen. Genet.* **101**:227–244.

126. Oh, E. Y., and L. Grossman. 1986. The effect of *Escherichia coli* Uvr protein binding on the topology of supercoiled DNA. *Nucleic Acids Res.* **14**:8557–8571.

127. Oh, E. Y., and L. Grossman. 1987. Helicase properties of the *Escherichia coli* UvrAB protein complex. *Proc. Natl. Acad. Sci. USA* **84**:3638–3642.

128. Oh, E. Y., and L. Grossman. 1989. Characterization of the helicase activity of the *Escherichia coli* UvrAB protein complex. *J. Biol. Chem.* **264**:1336–1343.

129. Oller, A. R., I. J. Fijalkowska, R. L. Dunn, and R. M. Schaaper. 1992. Transcription-repair coupling determines the strandedness of ultraviolet mutagenesis in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **89**:11036–11040.

130. Orren, D. K., and A. Sancar. 1989. The (A)BC excinuclease of *Escherichia coli* has only the UvrB and UvrC subunits in the incision complex. *Proc. Natl. Acad. Sci. USA* **86**:5237–5241.

131. Orren, D. K., and A. Sancar. 1990. Formation and enzymatic properties of the UvrB DNA complex. *J. Biol. Chem.* **265**:15796–15803.

132. Orren, D. K., C. P. Selby, J. E. Hearst, and A. Sancar. 1992. Post-incision steps of nucleotide excision repair in *Escherichia coli*. Disassembly of the UvrBC-DNA complex by helicase II and DNA polymerase I. *J. Biol. Chem.* **267**:780–788.

133. Paterson, M. C., B. P. Smith, and P. J. Smith. 1981. Measurement of enzyme-sensitive sites in UV- or γ-irradiated human cells using *Micrococcus luteus* extracts, p. 99–111. *In* E. C. Friedberg and P. C. Hanawalt (ed.), *DNA Repair—A Laboratory Manual of Research Procedures*. vol. 1, part A. Marcel Dekker, Inc., New York.

134. Pearlman, D. A., and S. R. Holbrook. 1985. Molecular models for DNA damaged by photoreaction. *Science* **227**:1304–1308.

135. Pedrini, A. M., and G. Ciarrochi. 1983. Inhibition of *Micrococcus luteus* DNA topoisomerase I by UV photoproducts. *Proc. Natl. Acad. Sci. USA* **80**:1787–1791.

136. Pettijohn, D. E., and P. C. Hanawalt. 1963. Deoxyribonucleic acid replication in bacteria following ultraviolet irradiation. *Biochim. Biophys. Acta* **72**:127–129.

137. Pettijohn, D. E., and P. C. Hanawalt. 1964. Evidence for repair-replication of ultraviolet damaged DNA in bacteria. *J. Mol. Biol.* **9**:395–410.

138. Pu, W. T., R. Kahn, M. M. Munn, and W. D. Rupp. 1989. UvrABC incision of N-methylmitomycin A-DNA monoadducts and cross-links. *J. Biol. Chem.* **264**:20697–20704.

139. Reardon, J. T., A. F. Nichols, S. Keeney, C. A. Smith, J. S. Taylor, and S. Linn. 1993. Comparative analysis of binding of human damaged DNA-binding protein (XPE) and *Escherichia coli* damage recognition protein (UvrA) to the major ultraviolet photoproducts: T[c,s]T, T[6-4]T, and T[Dewar]T. *J. Biol. Chem.* **268**:21301–21308.

140. Rothman, R. H. 1978. Dimer excision and repair replication patch size in *recL152* mutant of *Escherichia coli* K-12. *J. Bacteriol.* **136**:444–448.

141. Rothman, R. H., and A. J. Clark. 1977. Defective excision and postreplication repair of UV-damaged DNA in a *recL* mutant strain of *E. coli* K-12. *Mol. Gen. Genet.* **155**:267–277.

142. Rubin, J. S. 1988. The molecular genetics of the incision step in the DNA excision repair process. *Int. J. Radiat. Biol.* **54**:309–365.

143. Runyon, G. T., D. G. Bear, and T. M. Lohman. 1990. *Escherichia coli* helicase II (UvrD) protein initiates DNA unwinding at nicks and blunt ends. *Proc. Natl. Acad. Sci. USA* **87**:6383–6387.

144. Runyon, G. T., and T. M. Lohman. 1989. *Escherichia coli* helicase II (UvrD) protein can completely unwind fully duplex linear and nicked circular DNA. *J. Biol. Chem.* **264**:17502–17512.

145. Rupp, W. D., and P. Howard-Flanders. 1968. Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. *J. Mol. Biol.* **31**:291–304.

146. Rupp, W. D., A. Sancar, and G. B. Sancar. 1982. Properties and regulation of the UVRABC endonuclease. *Biochimie* **64**:595–598.

147. Sancar, A. 1987. DNA repair in vitro. *Photobiochem. Photobiophys.* **11**:301–315.

148. Sancar, A., N. D. Clarke, J. Griswold, W. J. Kennedy, and W. D. Rupp. 1981. Identification of the *uvrB* gene product. *J. Mol. Biol.* **148**:63–76.

149. Sancar, A., K. A. Franklin, and G. B. Sancar. 1984. *Escherichia coli* DNA photolyase stimulates uvrABC excision nuclease in vitro. *Proc. Natl. Acad. Sci. USA* **81**:7397–7401.

150. Sancar, A., and J. E. Hearst. 1993. Molecular matchmakers. *Science* **259**:1415–1420.

151. Sancar, A., and W. D. Rupp. 1983. A novel repair enzyme: UVRABC excision nuclease of *Escherichia coli* cuts a DNA strand on both sides of the damaged region. *Cell* **33**:249–260.

152. Sancar, A., and G. B. Sancar. 1988. DNA repair enzymes. *Annu. Rev. Biochem.* **57**:29–67.

153. Sancar, A., G. B. Sancar, W. D. Rupp, J. W. Little, and D. W. Mount. 1982. LexA protein inhibits transcription of the *E. coli uvrA* gene in vitro. *Nature* (London) **298**:96–98.

154. Sancar, A., and M. S. Tang. 1993. Nucleotide excision repair. *Photochem. Photobiol.* **57**:905–921.

155. Sancar, G. B., A. Sancar, J. W. Little, and W. D. Rupp. 1982. The *uvrB* gene of *Escherichia coli* has both lexA-repressed and lexA-independent promoters. *Cell* **28**:523–530.

156. Sancar, G. B., A. Sancar, and W. D. Rupp. 1984. Sequences of the *E. coli uvrC* gene and protein. *Nucleic Acids Res.* **12**:4593–4608.

157. Sauerbier, W., R. L. Millette, and P. B. Hackett, Jr. 1970. The effects of ultraviolet irradiation on the transcription of T4 DNA. *Biochim. Biophys. Acta* **209**: 368–386.

158. Schendel, P. F., M. Fogliano, and L. D. Strausbaugh. 1982. Regulation of the *Escherichia coli* K-12 *uvrB* operon. *J. Bacteriol.* **150**:676–685.

159. Schwartz, A. L., L. Marrot, and M. Leng. 1989. The DNA bending by acetylaminofluorene residues and by apurinc sites. *J. Mol. Biol.* **207**:445–450.

160. Seawell, P. C., and A. K. Ganesan. 1981. Measurement of strand breaks in supercoiled DNA by gel electrophoresis, p. 425–430. *In* E. C. Friedberg and P. C. Hanawalt (ed.), *DNA Repair—A Laboratory Manual of Research Procedures*. vol. 1, part B. Marcel Dekker, Inc., New York.

161. Sedgwick, B. 1989. In vitro proteolytic cleavage of the *Escherichia coli* Ada protein by the *ompT* gene product. *J. Bacteriol.* **171**:2249–2251.

162. Seeberg, E. 1978. Reconstitution of an *Escherichia coli* repair endonuclease activity from the separated *uvrA*$^+$ and *uvrB*$^+$/*uvrC*$^+$ gene products. *Proc. Natl. Acad. Sci. USA* **75**:2569–2573.

163. Seeberg, E. 1981. Multiprotein interactions in the strand cleavage of DNA damaged by UV and chemicals. *Prog. Nucleic Acid Res. Mol. Biol.* **26**:217–226.

164. Seeberg, E., and R. P. Fuchs. 1990. Acetylaminofluorene bound to different guanines of the sequence -GGCGCC- is excised with different efficiencies by the UvrABC excision nuclease in a pattern not correlated to the potency of mutation induction. *Proc. Natl. Acad. Sci. USA* **87**:191–194.

165. Seeberg, E., J. Nissen-Meyer, and P. Strike. 1976. Incision of ultraviolet-irradiated DNA by extracts of *E. coli* requires three different gene products. *Nature* (London) **263**:524–526.

166. Seeberg, E., and A. L. Steinum. 1982. Purification and properties of the uvrA protein from *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **79**:988–992.

167. Seeley, T. W., and L. Grossman. 1990. The role of *Escherichia coli* UvrB in nucleotide excision repair. *J. Biol. Chem.* **265**:7158–7165.

168. Selby, C. P., and A. Sancar. 1988. ABC excinuclease incises both 5′ and 3′ to the CC-1065-DNA adduct and its incision activity is stimulated by DNA helicase II and DNA polymerase I. *Biochemistry* **27**:7184–7188.

169. Selby, C. P., and A. Sancar. 1990. Molecular mechanisms of DNA repair inhibition by caffeine. *Proc. Natl. Acad. Sci. USA* **87**:3522–3525.

170. Selby, C. P., and A. Sancar. 1990. Structure and function of the (A)BC excinuclease of *Escherichia coli*. *Mutat. Res.* **236**:203–211.

171. Selby, C. P., and A. Sancar. 1990. Transcription preferentially inhibits nucleotide excision repair of the template DNA strand in vitro. *J. Biol. Chem.* **265**:21330–21336.

172. Selby, C. P., and A. Sancar. 1991. Gene- and strand-specific repair in vitro: partial purification of a transcription-repair coupling factor. *Proc. Natl. Acad. Sci. USA* **88**:8232–8236.

173. Selby, C. P., and A. Sancar. 1993. Molecular mechanism of transcription-repair coupling. *Science* **260**:53–58.

174. Selby, C. P., and A. Sancar. 1993. Transcription-repair coupling and mutation frequency decline. *J. Bacteriol.* **175**: 7509–7514.

175. Selby, C. P., E. M. Witkin, and A. Sancar. 1991. *Escherichia coli mfd* mutant deficient in "mutation frequency decline" lacks strand-specific repair: in vitro complementation with purified coupling factor. *Proc. Natl. Acad. Sci. USA* **88**:11574–11578.

176. Setlow, R. B., and W. L. Carrier. 1963. The disappearance of thymine dimers from DNA: an error-correcting mechanism. *Proc. Natl. Acad. Sci. USA* **51**:226–231.

177. Setlow, R. B., and J. D. Regan. 1981. Measurement of repair synthesis by photolysis of bromouracil, p. 307–318. *In* E. C. Friedberg and P. C. Hanawalt (ed.), *DNA Repair—A Laboratory Manual of Research Procedures*. Vol. 1, part B. Marcel Dekker, Inc., New York.

178. Setlow, R. B., P. A. Swenson, and W. L. Carrier. 1963. Thymine dimers and inhibition of DNA synthesis by ultraviolet irradiation of cells. *Science* **142**:1464–1466.

179. Sharma, S., T. F. Stark, W. G. Beattie, and R. E. Moses. 1986. Multiple control elements for the *uvrC* gene unit of *Escherichia coli*. *Nucleic Acids Res.* **14**:2301–2318.

180. Shi, Q., R. Thresher, A. Sancar, and J. Griffith. 1992. Electron microscopic study of (A)BC excinuclease. DNA is sharply bent in the UvrB-DNA complex. *J. Mol. Biol.* **226**:425–432.

181. Shi, Y. B., J. Griffith, H. Gamper, and J. E. Hearst. 1988. Evidence for structural deformation of the DNA helix by a psoralen diadduct but not by a monoadduct. *Nucleic Acids Res.* **16**:8945–8952.

182. Shimada, K., H. Ogawa, and J. Tomizawa. 1968. Studies on radiation-sensitive mutants of *E. coli*. II. Breakage and repair of ultraviolet irradiated intracellular DNA of phage lambda. *Mol. Gen. Genet.* **101**:245–256.

183. Shiota, S., and H. Nakayama. 1988. Evidence for a *Micrococcus luteus* gene homologous to *uvrB* of *Escherichia coli*. *Mol. Gen. Genet.* **213**:21–29.

184. Shiota, S., and H. Nakayama. 1989. *Micrococcus luteus* homolog of the *Escherichia coli uvrA* gene: identification of a mutation in the UV-sensitive mutant DB7. *Mol. Gen. Genet.* **217**:332–340.

185. Sibghat-Ullah, A. Sancar, and J. E. Hearst. 1990. The repair patch of *E. coli* (A)BC excinuclease. *Nucleic Acids Res.* **18**:5051–5053.

186. Siegel, E. C. 1973. Ultraviolet-sensitive mutator strain of *Escherichia coli* K-12. *J. Bacteriol.* **113**:145–160.

187. Sinden, R. R., and R. S. Cole. 1978. Repair of cross-linked DNA and survival of *Escherichia coli* treated with psoralen and light: effects of mutations influencing genetic recombination and DNA metabolism. *J. Bacteriol* **136**:538–547.

188. Sinden, R. R., and R. S. Cole. 1978. Topography and kinetics of genetic recombination in *Escherichia coli* treated with psoralen and light. *Proc. Natl. Acad. Sci. USA* **75**:2373–2377.

189. Sladek, F. M., B. Dynlacht, and P. Howard-Flanders. 1988. Characterization and partial purification of a novel activity from *E. coli* that acts on psoralen monoadducts. *J. Cell Biochem.* **12a**:276.

190. Smirnov, G. B., and A. G. Skavronskaya. 1971. Location of *uvr502* mutation on the chromosome of *Escherichia coli* K-12. *Mol. Gen. Genet.* **113**:217–221.

191. Smith, C. A., P. K. Cooper, and P. C. Hanawalt. 1981. Measurement of repair replication by equilibrium sedimentation, p. 289–305. *In* E. C. Friedberg and P. C. Hanawalt (ed.), *DNA Repair—A Laboratory Manual of Research Procedures*, vol. 1, part B. Marcel Dekker, Inc., New York.

192. Smith, C. A., and P. C. Hanawalt. 1978. Phage T4 endonuclease V stimulates DNA repair replication in isolated nuclei from ultraviolet-irradiated human cells, including xeroderma pigmentosum fibroblasts. *Proc. Natl. Acad. Sci. USA* **75**:2598–2602.

193. Snowden, A., Y. W. Kow, and B. Van Houten. 1990. Damage repertoire of the *Escherichia coli* UvrABC nuclease complex includes abasic sites, base-damage analogues, and lesions containing adjacent 5′ or 3′ nicks. *Biochemistry* **29**:7251–7259.

194. Sternglanz, R., S. DiNardo, K. A. Voelkel, Y. Nishimura, Y. Hirota, K. Becherer, L. Zumstein, and J. C. Wang. 1981. Mutations in the gene coding for *Escherichia coli* DNA topoisomerase I affect transcription and transposition. *Proc. Natl. Acad. Sci. USA* **78**:2747–2751.

195. Svoboda, D. L., C. A. Smith, J. S. Taylor, and A. Sancar. 1993. Effect of sequence, adduct type, and opposing lesions on the binding and repair of ultraviolet photodamage by DNA photolyase and (A)BC excinuclease. *J. Biol. Chem.* **268**:10694–10700.

196. Swenson, P. A., and R. B. Setlow. 1966. Effects of ultraviolet radiation on macromolecular synthesis in *Escherichia coli*. *J. Mol. Biol.* **15**:201–219.

197. Takagi, Y., M. Sekiguchi, S. Okubo, H. Nakayama, K. Shimada, S. Yasuda, T. Nishimoto, and H. Yoshihara. 1967. Nucleases specific for ultraviolet light-irradiated DNA and their possible role in dark repair. *Cold Spring Harbor Symp. Quant. Biol.* **33**:219–227.

198. Takahashi, M., E. Bertrand-Burggraf, R. P. Fuchs, and B. Norden. 1992. Structure of UvrABC excinuclease-UV-damaged DNA complexes studied by flow linear dichroism. DNA curved by UvrB and UvrC. *FEBS Lett.* **314**:10–12.

199. Tang, M. S., C. S. Lee, R. Doisy, L. Ross, D. R. Needham-VanDevanter, and L. H. Hurley. 1988. Recognition and repair of the CC-1065-(N3-adenine)-DNA adduct by the UVRABC nucleases. *Biochemistry* **27**:893–901.

200. Tang, M. S., and K. C. Smith. 1981. The effects of *lexA*, *recB21*, *recF143*, and *uvrD3* mutations on liquid-holding recovery in ultraviolet-irradiated *Escherichia coli* K12 *recA56*. *Mutat. Res.* **80**:15–25.

201. Tao, K., A. Noda, and S. Yonei. 1987. The roles of different excision-repair mechanisms in the resistance of *Micrococcus luteus* to UV and chemical mutagens. *Mutat. Res.* **183**:231–239.

202. Teo, I. A. 1987. Proteolytic processing of the Ada protein that repairs DNA O^6-methylguanine residues in *E. coli*. *Mutat. Res.* **183**:123–127.

203. Thiagalingam, S., and L. Grossman. 1991. Both ATPase sites of *Escherichia coli* UvrA have functional roles in nucleotide excision repair. *J. Biol. Chem.* **266**:11395–11403.

204. Thomas, D. C., T. A. Kunkel, N. J. Casna, J. P. Ford, and A. Sancar. 1986. Activities and incision patterns of ABC excinuclease on modified DNA containing single-base mismatches and extrahelical bases. *J. Biol. Chem.* **261**:14496–14505.

205. Thomas, D. C., M. Levy, and A. Sancar. 1985. Amplification and purification of UvrA, UvrB, and UvrC proteins of *Escherichia coli*. *J. Biol. Chem.* **260**:9875–9883.

206. Tomic, M. T., D. E. Wemmer, and S. H. Kim. 1987. Structure of a psoralen cross-linked DNA in solution by nuclear magnetic resonance. *Science* **238**:1722–1725.

207. Van den Berg, E. A., R. H. Geerse, J. Memelink, R. A. Bovenberg, F. A. Magnee, and P. Van de Putte. 1985. Analysis of regulatory sequences upstream of the *E. coli uvrB* gene: involvement of the DnaA protein. *Nucleic Acids Res.* **13**:1829–1840.

208. Van den Berg, E. A., R. H. Geerse, H. Pannekoek, and P. Van de Putte. 1983. In vivo transcription of the *E. coli uvrB* gene: both promoters are inducible by UV. *Nucleic Acids Res.* **11**:4355–4363.

209. Van de Putte, P., C. A. Van Sluis, J. Van Dillewijn, and A. Rörsch. 1965. The location of genes controlling radiation sensitivity in *Escherichia coli*. *Mutat. Res.* **2**:97–110.

210. Van Houten, B. 1990. Nucleotide excision repair in *Escherichia coli*. *Microbiol. Rev.* **54**:18–51.

211. Van Houten, B. 1994. Personal communication.

212. Van Houten, B., H. Gamper, S. R. Holbrook, J. E. Hearst, and A. Sancar. 1986. Action mechanism of ABC excision nuclease on a DNA substrate containing a psoralen crosslink at a defined position. *Proc. Natl. Acad. Sci. USA* **83**:8077–8081.

213. Van Houten, B., H. Gamper, A. Sancar, and J. E. Hearst. 1987. DNase I footprint of ABC excinuclease. *J. Biol. Chem.* **262**:13180–13187.

214. Van Houten, B., S. Illenye, Y. Qu, and N. Farrell. 1993. Homodinuclear (Pt, Pt) and heterodinuclear (Ru, Pt) metal compounds as DNA-protein cross-linking agents: potential suicide DNA lesions. *Biochemistry* **32**:11794–11801.

215. Van Houten, B., and A. Snowden. 1993. Mechanism of action of the *Escherichia coli* UvrABC nuclease: clues to the damage recognition problem. *Bioessays* **15**:51–59.

216. Van Sluis, C. A., L. E. Mattern, and M. C. Paterson. 1974. Properties of *uvrB* mutants of *Escherichia coli* K12. I. Effects of UV irradiation on DNA metabolism. *Mutat. Res.* **25**:273–279.

217. Varghese, A. J., and R. S. Day. 1970. Excision of cytosine-thymine adducts from the DNA of ultraviolet-irradiated *Micrococcus radiodurans*. *Photochem. Photobiol.* **11**:511–517.

218. Visse, R. M., M. De Ruijter, G. F. Moolenaar, and P. Van de Putte. 1992. Analysis of UvrABC endonuclease reaction intermediates on cisplatin-damaged DNA using mobility shift gel electrophoresis. *J. Biol. Chem.* **267**:6736–6742.

219. Visse, R. M., M. De Ruijter, M. Ubbink, J. A. Brandsma, and P. Van de Putte. 1993. The first zinc-binding domain of UvrA is not essential for UvrABC-mediated DNA excision repair. *Mutat. Res.* **294**:263–274.

220. Voight, J. M., B. Van Houten, A. Sancar, and M. D. Topal. 1989. Repair of O^6-methylguanine by ABC excinuclease of *Escherichia coli*. *J. Biol. Chem.* **264**:5172–5176.

221. von Hippel, P. H., and O. G. Berg. 1989. Facilitated target location in biological systems. *J. Biol. Chem.* **264**:675–678.

222. Wahl, A. F., J. W. Hockensmith, S. Kowalski, and R. A. Bambara. 1983. Alternative explanation for excision repair deficiency caused by the *polAex1* mutation. *J. Bacteriol.* **155**:922–925.

223. Walker, J. E., M. Saraste, M. J. Runswick, and N. J. Gay. 1982. Distantly related sequences in the α- and β-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J.* **1**:945–951.

224. Wang, J., and L. Grossman. 1993. Mutations in the helix-turn-helix motif of the *Escherichia coli* UvrA protein eliminate its specificity for UV-damaged DNA. *J. Biol. Chem.* **268**:5323–5331.

225. Washburn, B. K., and S. R. Kushner. 1991. Construction and analysis of deletions in the structural gene (*uvrD*) for DNA helicase II of *Escherichia coli*. *J. Bacteriol.* **178**:2569–2575.

226. Weiss, B., and L. Grossman. 1987. Phosphodiesterases involved in DNA repair. *Adv. Enzymol. Relat. Areas Mol. Biol.* **60**:1–34.

227. Witkin, E. M. 1956. Time, temperature, and protein synthesis: a study of ultraviolet-induced mutation in bacteria. *Cold Spring Harbor Symp. Quant. Biol.* **21**:123–140.

228. Witkin, E. M. 1966. Radiation-induced mutations and their repair. *Science* **152**:1345–1353.

229. Yang, Y., and G. F. Ames. 1988. DNA gyrase binds to the family of prokaryotic repetitive extragenic palindromic sequences. *Proc. Natl. Acad. Sci. USA* **85**:8850–8854.

230. Yeung, A. T., W. B. Mattes, E. Y. Oh, and L. Grossman. 1983. Enzymatic properties of purified *Escherichia coli* uvrABC proteins. *Proc. Natl. Acad. Sci. USA* **80**:6157–6161.

231. Yeung, A. T., W. B. Mattes, E. Y. Oh, and L. Grossman. 1983. Enzymatic properties of the purified *Escherichia coli* uvrABC complex, p. 77–86. *In* E. C. Friedberg and B. A. Bridges (ed.), *Cellular Responses to DNA Damage*. Alan R. Liss, Inc., New York.

232. Yeung, A. T., W. B. Mattes, E. Y. Oh, G. H. Yoakum, and L. Grossman. 1986. The purification of the *Escherichia coli* UvrABC incision system. *Nucleic Acids Res.* **14**:8535–8556.

233. Youngs, D. A., E. Van der Schueren, and K. C. Smith. 1974. Separate branches of the uvr gene-dependent excision repair process in ultraviolet-irradiated *Escherichia coli* K-12 cells: their dependence upon growth medium and the *polA*, *recA*, *recB*, and *exrA* genes. *J. Bacteriol.* **117**:717–725.